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THE EFFECT OF POSTERIOR HYPOPHYSEAL EXTRACT ON THE RETENTION OF WATER AND SALT INJECTED INTO FROGS[1]

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When frogs are almost completely immersed in water and injected with pituitrin, they take up 15 to 20 per cent of their weight of water in 2 to 4 hours and their weight does not return to the initial for 6 to 8 hours at room temperature and at seasons when they do not ordinarily hibernate (2). To date there is evidence for and no evidence against this reaction being almost if not completely extra-renal in origin (10). Boyd and Whyte (3) concluded, assuming pituitrin "alters the physiology of the skin," that the hormone must *a*, inhibit loss of water through the skin, and *b*, excite an increased uptake of water through the skin but that mechanism (*b*) need not be advanced could it be shown that frogs *in water* are capable of taking up and losing 15 to 20 per cent of their weight of water within three hours.

The present experiments were concerned with further studies on the mechanism of the action of pituitrin on the water balance of frogs. In the first place, conclusion (a) of Boyd and Whyte (3) does not strictly apply to the experiment described because Boyd and Whyte (3), to be exact, showed that pituitrin inhibited the loss of *normal* body water. The first question investigated was, thus, the effect of pituitrin on *extra* water added to the body of frogs.

The effect of pituitrin on pituitrin-added body water. Extra water was added to the body of frogs (Rana pipiens) by placing them in water and injecting 0.5 international unit of Pituitrin Surgical (supplied in generous quantities by Dr. E. A. Sharp of Parke, Davis & Co.) per 10 grams body weight using the technique of Boyd and Brown (2). When the frogs had taken up approximately 15 per cent of their weight of extra water, they

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were again injected with the same dose of pituitrin and this was repeated at 2 to 3 hour intervals to see if the augmented weight could be maintained. The mean results obtained in 24 frogs have been plotted in figure 1 together with a control series of 24 frogs receiving no pituitrin.

Repeated injections of pituitrin enabled the frogs to take up and hold for at least 12 hours (the duration of the experiment) 15 to 20 per cent of their weight of added water. The animals gradually became less active than usual and this effect may be likened unto "water intoxication" produced in dogs by Weir, Larson and Rowntree (11) through repeated administration of water and posterior lobe extract and in epileptic patients by McQuarrie and Peeler (7) and Clegg and Thorpe (4). The common

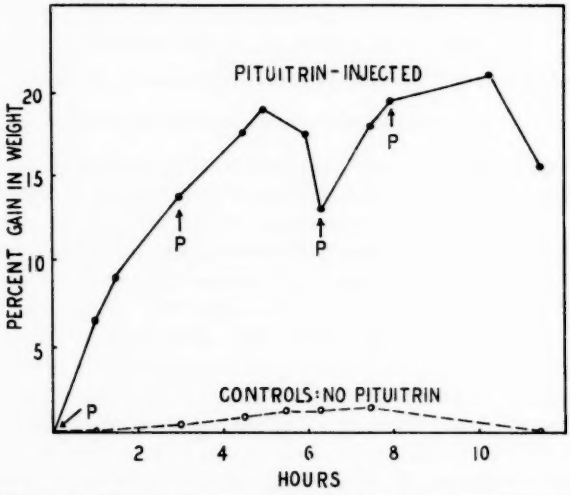

Fig. 1. The effect of repeated injections of pituitrin *P*, on the uptake and retention of water by frogs in water.

interpretation of water intoxication in mammals so produced is that pituitary extracts cause an antidiuresis by acting upon the kidney. These results in frogs suggest that the mechanism may be extra-renal as well as, or even rather than, renal.

The results depicted in figure 1 admit two interpretations. Pituitrin may either *a*, have continued to excite increased water uptake through the skin (assuming again the usual interpretation), or *b*, have inhibited the loss of added water through the skin.

The effect of pituitrin on injected water. In order to eliminate any hypothetical stimulating effect of pituitrin on water uptake frogs were taken out of water and injected. In this and in subsequent experiments, the general technique followed was that of Boyd and Whyte (3) and from

6 to 60 frogs were used to determine the mean effect of each factor. Frogs were injected into the dorsal lymph sac with 10 per cent of their weight of distilled water, more than 10 per cent tending to leak out through the opening made for injection. At the same time one group was injected intramuscularly with 0.5 international unit of pituitrin per 10 grams body weight and a control group received no pituitrin. Two other control groups received no distilled water, one getting pituitrin and one not. The mean changes in weight were expressed as loss of weight in grams per 100 grams of frogs' initial weight, the initial weight being that before the animals received any injection. This loss in weight per hour represents loss of normal body water plus loss of injected water if water was injected. The loss of normal body water was practically identical to that previously

TABLE 1

The effect of pituitrin on the retention of extra water added to the body of the frog

	GRAMS OF WATER LOST PER 100 GRAMS OF FROG					
	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours
Receiving no pituitrin						
10 per cent water injected	1.5	3.0	5.2	7.3	9.1	10.4
No water injected	1.3	2.5	3.4	4.5	5.8	6.8
Loss of injected water	0.2	0.5	1.8	2.8	3.3	3.6
Hourly rate of loss of extra water	2%	3%	13%	10%	5%	3%
Receiving pituitrin						
10 per cent water injected	1.0	1.8	2.7	4.2	5.7	7.2
No water injected	1.4	2.2	2.6	3.1	3.7	4.7
Loss of injected water	0.0	0.0	0.1	1.1	2.0	2.5
Hourly rate of loss of extra water	0%	0%	1%	10%	9%	5%
Hourly rate at which added water is being retained by pituitrin	+100%	+100%	+92%	0%	−80%	−67%

reported (3). The results of this experiment have been assembled in table 1 in which each mean represents the average of at least 30 frogs.

When water was injected into a frog, the subsequent hourly loss of water was greater than normal. When pituitrin was injected into a frog, the subsequent hourly loss of water was less than normal. When water and pituitrin were simultaneously injected, the hourly loss of water was no greater than when pituitrin was given alone. This may be taken to indicate that under the conditions of this experiment, pituitrin completely inhibited the loss of added body water and, as shown in the last line of table 1, the inhibition lasted for a period of three hours. Following this, there was an out-pouring of the retained water in excess of normal and this began to abate in the 6th hour. The net effect after 6 hours was that

pituitrin had still retained some of the added water which amounted to about $\frac{1}{3}$ more than would be expected at this time without pituitrin.

It may be concluded that pituitrin inhibits completely the loss of extra water injected into frogs under these conditions for a period of 3 hours. This interval corresponds exactly with that period during which frogs in water and injected with pituitrin take up and hold water. If, therefore, it could be shown that frogs in water are capable of maintaining a dynamic equilibrium between water outside and water inside the body such that water up to the extent of 15 to 20 per cent of the weight of the frogs could be taken up and lost within three hours, then the simpler explanation of the effect of pituitrin might be offered, to wit, that pituitrin simply inhibits the loss of water by frogs in water and as a result water entering the body through the skin, etc., is retained for a period of 3 hours.

The water exchange of frogs in water. In order to obtain information on how rapidly frogs in water may take up water, 24 frogs were dehydrated in a forced draft until they had lost a mean of 16.2 per cent of their weight of body water which, with the draft used, required two hours. They were then placed in water and in 1 hour had regained 45 per cent, and in two hours 85 per cent of the water lost. In other words, they had taken up practically 14 per cent of their original weight of water within 2 hours. It may be concluded that frogs are capable of taking up 15 per cent of their weight of water within 3 hours.

To measure the time required for frogs in water to lose 15 to 20 per cent of their weight of water, 60 frogs were placed in water and injected with pituitrin in the refrigerator room. They took up 22 per cent of their weight of water and held it for 10 to 14 hours after which they began to slowly lose weight, indicating that the effect of pituitrin was slowly wearing off as it does at these low temperatures (2). They were then transferred to room temperature in water and within 2 hours had lost 75 per cent of the water previously held in the refrigerator room. In other words, frogs are capable of losing 15 to 20 per cent of their weight of water within 3 hours.

These experiments therefore demonstrate that frogs in water can take up and lose 15 to 20 per cent of their weight of water within a period of 3 hours. The experiments were not done under conditions entirely analogous to that of normal frogs in water, but they suggest that the action of pituitrin upon frogs in water is solely to inhibit the loss of water from the body.

In conjunction with the previous results of Boyd and Whyte (3), these experiments suggest further that when frogs are taken out of water, some mechanism comes into force whereby they do not lose water as rapidly as when in water, providing they are not placed in a draft of air or in a warm environment. Since frogs are usually considered as being in a state of continuous diuresis when in water, then removing frogs from

water may be considered analogous to dehydrating mammals. If so, then the removal of frogs from water may bring into effect an altered function of their posterior hypophysis, since Gilman and Goodman (5) found more antidiuretic substance in the urine of dehydrated (thirsted) than of normal, non-dehydrated animals.

The comparative effect of pituitrin, pitressin and pitocin. What is the relative power of pituitrin, pitressin and pitocin to inhibit the loss of water injected into the body of a frog? Experiments were assembled with 4 groups of 6 frogs each. One group of frogs received only 10 per cent of their weight of distilled water, a second group water plus 0.2 unit of pituitrin per 10 grams, a third group water plus 0.2 international unit of pitocin and a fourth group water plus 0.2 pressor unit of pitressin. The animals were weighed out of water until their weight had returned to the initial before injecting water. A total of 7 comparisons, each containing 24 frogs, unanimously demonstrated that pitocin and pituitrin were equally effective dose for dose in inhibiting loss of injected water and that both were superior to pitressin, international dose for pressor dose. Compared with the control receiving only an injection of water, pitressin prolonged the time required to reach the initial weight by an average of 3.9 per cent, pitocin 26.7 per cent and pituitrin 27.7 per cent.

These results suggest that the factor responsible for the inhibition of loss of extra water is contained chiefly in the pitocin fraction of pituitary extracts. Boyd and Brown (2) found that 1 international unit of pitocin was more effective than 8 pressor units of pitressin in causing frogs to take up water. About the same relationship existed in the present experiments, 1 international unit of pitocin prolonging the retention of injected water about 8 times as long as 1 pressor unit of pitressin. This suggests that the same principle of pituitary extracts is involved in the two reactions.

The injection of solutions of sodium chloride. The above experiments on pitocin and pitressin emphasize a characteristic difference between the extra-renal response of frogs to posterior hypophyseal extracts and the (supposedly) renal response of mammals. As a result of a number of experiments in mammals (for example 1, 6, 8, 9), there has arisen the conception that pituitrin has a dual action in mammals: *a*, a "water retaining" action, and *b*, a "salt mobilizing" action (8) which find expression in a tendency to inhibit water diuresis but to stimulate excretion of salt. As the amount of salt is increased in administered water, pituitrin has progressively less of a water diuresis inhibiting action. A point is finally reached at which pituitrin does not affect the volume of urine formed and the administration of a more concentrated salt solution leads to a diuresis when pituitrin is injected, water being necessary for the greater excretion of salt brought about by the action of pituitrin.

It was decided to investigate if pituitrin has a "salt mobilizing" action in frogs and if so is it through the function of the same principle which causes water retention. Groups of 24 frogs received into the dorsal lymph sac, 10 per cent of their weight of solutions in distilled water of sodium chloride from 0 to 1.0 per cent and 0.2 unit of pituitrin, or pitocin or pitressin per 10 grams with controls receiving no pituitary extract. The posterior hypophyseal extracts had progressively less power to inhibit water loss as more and more sodium chloride was added to the injected water and at 0.7 per cent concentration they had no appreciable effect upon the loss of weight of the frogs. When extracts were injected with solutions containing from 0.7 to 1.0 per cent of sodium chloride, they actually stimulated loss of water and pitressin was more active pressor dose for international dose, than either pitocin or pituitrin. These results prove that the administration of sodium chloride with water can inhibit and, in sufficient amount, reverse the power of extracts of the posterior hypophysis to inhibit loss of water injected into frogs.

The salt excretory action of pitressin. While pitressin has less power than pituitrin or pitocin to inhibit loss of injected water in frogs, it apparently has more power to excite excretion of sodium chloride. The finding appeared worthy of more detailed study. Frogs were injected with 10 per cent of their weight of 1 per cent sodium chloride and varying doses of pituitrin, pitressin and pitocin with controls receiving no extract. They were periodically weighed until their weight had returned to the initial before receiving an injection. In 29 of 30 experiments performed, the pituitary extracts produced an increased rate of water loss. The results are summarized in table 2.

Pituitrin shortened the time required to reach the initial weight by a maximum of 40 per cent with a dose of 0.005 international unit per gram. Without exception, decreasing the dose of pituitrin from 0.5 to 0.005 unit per 10 grams the more effective it was in stimulating the excretion of water and salt. It is presumed that pituitrin was stimulating excretion of salt because it inhibits excretion of water and hence the excessive loss of water when 1 per cent saline was injected most likely represented water required to dissolve excreted salt.

These results suggest that there are two principles in pituitrin, one which inhibits water loss and one which stimulates excretion of salt in frogs. The optimal dose of the former is contained in that volume of Pituitrin Surgical which contains 0.2 international unit of posterior pituitary extract and the optimal dose per 10 grams of frog of the latter is contained in 1/40th of this volume. Hence when 1 per cent saline is injected with pituitrin, as the dose of pituitrin is decreased from 0.2 unit a smaller percentage of the optimal dose of the water retention principle is present but still an optimal dose of the salt excretion principle and thus less water is retained and more water excreted as a solvent for the salt.

Pitocin shortened the time required to reach the initial body weight in 1 per cent saline injected frogs by a maximum of 21 per cent with a dose containing 0.1 international unit per 10 grams. Pitocin was less effective in stimulating salt excretion than pituitrin or pitressin.

Pitressin under the same conditions increased the rate of water loss by a maximum of 53 per cent, the optimal dose being contained in that volume which had 0.3 pressor unit per 10 grams. Pitressin had the greatest stimulating effect on salt excretion. Since pitressin is standardized in pressor units and pituitrin and pitocin in international oxytocic units, it is impossible to compare directly the doses employed. However, the optimal salt excretory effect of pitressin was $2\frac{1}{2}$ times the optimal effect which could be obtained with pitocin. The apparent presence of

TABLE 2

The effect of varying doses of pitressin, pituitrin and pitocin on the interval required for frogs, out of water and injected with 10 per cent of their weight of 1 per cent sodium chloride, to reach their initial weight before injection

Each interval figure represents the mean of at least six frogs

PITRESSIN		PITUITRIN		PITOCIN	
Dose	Interval	Dose	Interval	Dose	Interval
Pressor U./10 gm.	*hours*	*Int. U./10 gm.*	*hours*	*Int. U./10 gm.*	*hours*
None	6.0	None	5.9	None	5.6
0.0025	5.3	0.0025	4.9	0.0025	5.5
0.005	5.3	0.005	**3.6**	0.005	5.3
0.01	5.0	0.01	3.8	0.01	6.0
0.025	4.9	0.025	3.6	0.025	5.2
0.05	4.5	0.05	4.1	0.05	4.9
0.1	4.2	0.1	4.0	0.1	**4.4**
0.15	3.8	0.2	5.1	0.2	4.6
0.3	**2.85**	0.5	5.5	0.5	5.1
0.5	3.3				
1.0	3.8				

such large amounts of the salt excreting principle in pitressin may explain why it is usually impossible to obtain as much water retention in frogs with pitressin as with pitocin or pituitrin even though very large doses be used (2). Conversely, the presence of large amounts of the water retention principle in pitocin may in turn prevent it from exhibiting as much salt excretory stimulation as pitressin.

SUMMARY

By taking frogs out of water and injecting into the dorsal lymph sac 10 per cent of their weight of distilled water or solutions of sodium chloride, it was found:

1. Pituitrin completely inhibits the loss of added distilled water for 3 hours.

2. Pitocin and pituitrin are equally effective in inhibiting loss of added water and pitressin is less effective.

3. Sodium chloride added to the injected water decreases the water retaining power of pituitary extracts and reverses their action when the concentration of sodium chloride exceeds 0.7 per cent.

4. The optimal salt excretory dose of pituitrin is 40 times less than the optimal water retention dose.

5. Pitressin is more effective than pituitrin or pitocin in stimulating salt excretion.

From these and related experiments, it was concluded:

1. Pituitrin inhibits the loss of normal and of added body water of frogs in water and probably does not excite an increased rate of absorption of water.

2. Pituitrin probably contains two amphibian water balance principles, one causing water retention and the other stimulating salt excretion.

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THE NATURE OF THE FACTOR WHICH IS CONCERNED IN LOSS OF BLOOD COAGULABILITY OF BILE FISTULA AND JAUNDICED RATS

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Bile fistula and jaundiced animals often exhibit a marked hemorrhagic tendency subsequent to trivial injuries. This condition is associated with a decrease in the blood prothrombin level (1 to 5 inc.). A similar tendency is often seen in human patients suffering from obstructive jaundice (6 to 11 inc.). The administration of vitamin K concentrates will correct the condition (2, 5, 8 and 9). The subject has recently been reviewed (11). Bile is essential to the intestinal absorption of adequate amounts of vitamin K in the rat (2), man (9, 15) and dog (16). The present work represents an extension of previous work[1] (2). In the present series the effect of various dietary supplements upon the bleeding tendencies of bile fistula and jaundiced rats, maintained either on normal or vitamin K-free diets, has been studied. The original work has been confirmed and extended using animals maintained on vitamin K-free diets.

METHODS. Bile fistulas were established in a series of rats[2] by the technique previously described (12). The animals rapidly recovered from the effects of the operation and in most cases showed body weight increases during the experimental period. After the operation part of the animals were continued on our stock diet[3] and the remaining animals were trans-

[1] A brief preliminary report covering the portion of this work which was conducted in the department of Biochemistry at the University of California has been published (2). The author gratefully acknowledges the interest and coöperation extended by Dr. C. L. A. Schmidt of the University of California and Dr. P. A. Shaffer of the Washington University School of Medicine during the course of this work.

[2] Rats raised from the Evans California Strain as well as animals obtained from Anheuser-Busch have been used in these studies. The animals used varied in body weight from 100 to 300 grams.

[3] Ground wheat 30 lbs., whole milk powder 10 lbs., alfalfa meal 1 lb., fish meal 5 lbs., sodium chloride and calcium carbonate each 100 grams. The diet was supplemented with a concentrate of vitamins A and D prepared from cod liver oil. The concentrates were injected subcutaneously or fed with bile salt supplements at weekly intervals.

ferred to a modified Almquist vitamin K-free diet (13).[4] The animals were housed in individual screen-bottom cages.

Periodically at 7 to 10 day intervals after the operation blood samples were taken by heart puncture (under light ether anesthesia) and the blood prothrombin content was determined by the method of Quick[5] (14) as previously described (2). Clotting times were determined by the capillary tube method of Duke. Blood was obtained by clipping off a small piece of the end of the tail. The blood prothrombin level was followed until values of 30 to 40 per cent of normal or lower were recorded. The animals were then treated with the various supplements.[6] The response of the blood prothrombin level to treatment, together with changes in the bleeding tendencies of the animals, was used to indicate the effectiveness of the treatment under test. All experiments were conducted at least in duplicate and in many cases 10 to 15 or more animals have been employed in a single instance.

Results. *Bile fistula series.* The prothrombin level fell progressively following the operation. The rate of fall varied considerably from one animal to another but values below 30 per cent of normal were recorded within 14 to 60 days after the operation in most of the 80 cases studied. The rate of fall of the blood prothrombin in the group maintained on the stock diet and those given the vitamin K-free diet were not significantly different.

The bile fistula animals were in a good state of health and appeared normal for several weeks after the operation. The bleeding tendency usually exhibited itself after some injury. A minor scratch or irritation would often lead to extensive hemorrhage. Animals have bled to death from injuries inflicted by marking the ears with a paper punch, or clipping the end of the tail with scissors. Extensive hemorrhages have been observed in the thoracic cavity, the cranial vault, and the gastro-intestinal tract; any trivial injury may jeopardize the life of the animal. Animals with low blood prothrombin levels would often fail to stop bleeding for

[4] Fish meal (ether or acetone-alcohol extracted) 12 per cent, yeast (ether extracted) 10 per cent, ground rice 73 per cent, Osborne-Mendel salt mixture 4 per cent. Vitamins A, D and E were supplied as the non-saponifiable fraction of cod liver oil and wheat germ oil equivalent to 1 per cent of each oil in the diet. The supplements were injected subcutaneously or fed with bile salts weekly during the experiments.

[5] The good correlation between the clinical and chemical findings obtained in this series, together with the small amount of blood available seems to the author to justify the use of the less specific but more economical Quick method.

[6] The vitamin K concentrate kindly furnished by Dr. H. J. Almquist was administered in a 5 mgm. dose daily with 100 mgm. of crystalline bile salts isolated from beef bile. The vitamin K concentrate when fed to chicks on a vitamin K-free diet at a dosage level of 10 mgm. per kilogram of diet prevented all hemorrhagic tendencies.

periods as long as 24 to 36 hours after clipping the end of the tail. In these cases clotting times were often increased. Values of 3 to 9 minutes have been recorded as compared with normal values of 1 to 1½ minutes. As a rule the bleeding tendency does not develop until after the blood prothrombin level has fallen to levels of 30 per cent of normal or less. Animals with very low blood prothrombin levels appeared normal in all other respects. Animals with prothrombin levels below 25 per cent frequently bled to death from the heart puncture. For this reason treatment was usually initiated when the blood prothrombin level reached 30 to 40 per cent of normal. The animals responded to treatment by a rise in the blood prothrombin level and a decrease in the bleeding time within a 2 to 5 day period. The mortality following heart puncture in operated animals treated with vitamin K concentrates or normal animals was negligible.

After treatment with active extracts was discontinued the blood prothrombin would often remain near normal for a week or so, then fall again, while in other cases a rapid decline followed when treatment was discontinued. When inactive extracts were administered no change or a decrease in the blood prothrombin level was noted at the time the next determination was carried out. The process of depletion, correction of the condition by active extracts, followed by a second depletion, etc., was carried out repeatedly in many cases. Frequently fatal hemorrhages from the heart puncture wound resulted after treatment with inactive extracts.

The various preparations found to be active in bile fistula rats maintained on our stock diet were beef bile, sheep bile, boiled bile, crystalline bile salts, bile boiled with alkali, hexane extract of alfalfa, and vitamin K concentrates administered orally, in great excess, or in smaller quantities with bile salts. Vitamin K concentrates administered intraperitoneally or subcutaneously were also active. Ether extract of acid bile, lemon juice with its content of vitamins C and P, vitamin K concentrates treated with alkali, and ether extract of urine were in all cases inactive. Calcium carbonate, methyl linolate, and vitamins A, D, and E administered in large amounts were without effect. Massive doses of the latter four were also injected subcutaneously without result. Similar results were obtained in the group of bile fistula animals maintained on vitamin K-free diets. In this case, however, bile salts were found to be inactive. The activity due to bile salt administration to the bile fistula rats on a normal diet must have been due to increased intestinal absorption of the vitamin K contained in the diet used. Bile obtained from both beef and sheep gall bladders proved effective at a 2.0 cc. per day level. Prolonged dialysis of bile at 0°C. in a parlodion membrane did not decrease the effectiveness of bile in preventing the syndrome. Vitamin K concentrates treated with alkali were inactive. Bile treated with alkali retained its potency. This

discrepancy has not been explained. Bile when fed to vitamin K deficient chicks exerted some protective action. Bile, however, is not a good source of vitamin K. The possibility of the bacterial synthesis of the small amount of vitamin K found in the bile has not been entirely removed. The bile was obtained from the slaughter house in a fresh condition and stored in the frozen state until used. The active factor in bile is removed by zinc hydroxide precipitation, and can be demonstrated in the precipitate after removal of the zinc. The following facts also strongly indicate the effectiveness of bile in the cure and prevention of the condition. Animals raised on the stock diet for many generations do not develop any hemorrhagic tendency. Within a few weeks after altering the biliary drainage into the intestine, either by establishing bile fistulas or ligating and sectioning the bile duct, the bleeding tendency exhibits itself. By the administration of bile at weekly or semi-weekly intervals it has been possible to control or prevent the hemorrhagic tendency for periods of time as long as 2 years after the bile fistula operation (17). Vitamin K concentrates are fully as effective if administered with bile salts.

Jaundiced series. Two series of experiments have also been conducted in rats jaundiced by double ligation and section of the common bile duct. One series of 47 animals were maintained on the stock diet while the second series were transferred to the vitamin K-free diet after the operation. Three to seven weeks after the operation prolonged bleeding times and low blood prothrombin values associated with an increased hemorrhagic tendency was evident in about half of the animals. Many of the animals died before the tendency developed. The rate of fall of the blood prothrombin was quite variable but the trend in all cases was definitely downward. In many cases an initial rise in the blood prothrombin curve preceded the final drop. Those animals with prothrombin values below 30 per cent usually exhibited increased hemorrhagic tendencies. Vitamin K concentrates administered parenterally, or orally with bile salts, corrected the condition, the latter being the more effective. The response was slower than had been the case in the bile fistula series. No significant difference was noted in the rate of fall of the blood prothrombin levels of the animals on the two diets.

In three cases the jaundiced condition cleared up spontaneously after low blood prothrombin values had been observed. In each case the blood prothrombin returned to normal. At autopsy it was found that the bile was again draining into the duodenum. A similar finding was observed in one case that had been maintained on the vitamin K-free diet. In this case a period of 4 weeks elapsed between the time the icteric condition disappeared and the prothrombin level returned to the normal. This occurrence was subsequently explained as due to bacterial synthesis

of small amounts of vitamin K in the intestinal tract (18). The amounts made available in this manner seem to be adequate to protect a large percentage of normal rats, raised on a vitamin K-free diet, from hemorrhagic tendencies. It is, however, not enough to be effective within a period of a week, when rendered available by feeding bile salts to the jaundiced or bile fistula rat exhibiting bleeding tendencies when maintained on the vitamin K-free diet.

The parenteral administration of vitamin K concentrates corrects or controls the hemorrhagic tendencies of jaundiced rats. This treatment in both the bile fistula and the jaundiced animals is more effective than bile feeding or other types of treatment. Animals raised for extended periods of time on vitamin K-free diets usually exhibit hemorrhagic symptoms within a week, after the bile fistula or jaundiced condition is established (18). The delayed occurrence of the syndrome in animals maintained on normal stock diets up to the time of operation, is probably due to body stores of the factor which must be depleted before the hemorrhagic tendency develops.

SUMMARY

Bile fistula or jaundiced animals exhibit marked bleeding tendencies. This tendency is associated with an increased bleeding time and a low blood prothrombin value. It can be cured by the administration of vitamin K concentrates. Bile salts are essential to the intestinal absorption of vitamin K in the rat. Bile salts when administered to animals raised on vitamin K-free diets are much less efficient than when administered along with vitamin K concentrates.

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STUDIES ON THE VITAMIN K REQUIREMENTS OF THE RAT

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Almquist and Stokstad (1) in this country, and Dam and Schønheyder (2 to 5 inc.) working independently in Denmark, discovered and described a hemorrhagic disease in chickens which is due to a dietary deficiency of a fat soluble factor, now called vitamin K (2) (3). The absence of this factor in the diet of the chick results in a low blood prothrombin level which can be correlated with the bleeding tendencies of such animals (6). The disease has been produced in chickens, ducklings, and young geese. It has been seen only in a mild form in pigeons and canaries raised on vitamin K-free diets. Attempts to produce the disease in rats, guinea pigs, and dogs have been unsuccessful (7).

Previous work by the author (8) has demonstrated that the hemorrhagic condition, associated with low blood prothrombin values, often seen in bile fistula or jaundiced rats can be cured or prevented by vitamin K concentrates, administered parenterally or orally with bile salts. This work also demonstrated the necessity of bile salts for the intestinal absorption of vitamin K in the rat. Recent publications indicate that vitamin K concentrates are active in a similar manner in the jaundiced or bile fistula patients (9) and in bile fistula dogs (10). The prevalence of the hemorrhagic syndrome in the rat, dog, and human patient when biliary drainage into the intestinal tract has been altered, the necessity of bile salts in the intestinal absorption of vitamin K in these species, and the cure or prevention of the hemorrhagic syndrome by administration of vitamin K concentrates, all these facts point to the conclusion that vitamin K probably has a rôle in a variety of species. There is, however, no direct evidence in the literature to indicate that normal animals other than birds require the factor. The present work is an attempt to explain this discrepancy by producing vitamin K deficiency symptoms in the normal rat by dietary means.

METHODS. Rats 28 days of age, with body weights between 50 and 60 grams, were transferred to a modification of the Almquist vitamin K-free basal diet (see page 430).

Modification of Almquist vitamin K-free basal diet

Fish meal, ether extracted[1]	12 per cent
Yeast, ether extracted	10 per cent
Ground rice	73 per cent
Osborne-Mendel salt mixture	4 per cent

Vitamins A, D and E were supplied as the nonsaponifiable fraction of cod liver oil[2] and wheat germ oil, in amounts equivalent to 1 per cent of each oil in the diet. The supplements were added directly to the diet twice weekly. Linoleic acid, isolated from corn oil as the tetrabromide and converted into methyl linolate by means of zinc and hydrogen chloride in methyl alcohol, was fed at a dosage level of 0.4 cc. per rat per week. Distilled water was given ad libitum. The animals were housed in screen-bottom cages, two to three animals per cage. At the time the animals attained sexual maturity they were mated and a second and third generation of rats were raised on the above diet. Each new batch of diet used, with all supplements added, was checked for the absence of vitamin K by feeding it to young chicks. All batches of the diet used in these studies consistently produced the disease in the chicks within a 2–3 week period. In all, 37 first generation, 26 second generation, and 14 third generation rats have been maintained on the above diet for periods of time ranging from 40 to 320 days. Periodically during the experiment blood samples were taken via heart puncture and the blood prothrombin values were determined by the method of Quick (11). Clotting and bleeding times were also determined periodically in many cases.

Results. Twelve of the 77 animals used in these experiments exhibited prothrombin values below 50 per cent of normal, four showing values of 5, 25, 25, and 30 per cent at some time during the depletion period.[3] Six of these twelve animals having low blood prothrombin values were treated with a vitamin K concentrate.[4] The bleeding times which had been prolonged to several hours in these cases promptly returned to normal. The prothrombin level of the blood rose promptly and in several cases remained at a normal level for the duration of the experiment (30–70 days). In 3 cases the blood prothrombin value again fell to low levels 4 to 5 weeks after the vitamin K concentrate was administered.

[1] In some cases alcohol-acetone extracted fish meal was employed.

[2] The cod liver oil and wheat germ oil used in these studies were kindly supplied by E. R. Squibb and Sons.

[3] Blood platelet counts made on these animals as well as on bile fistula animals with bleeding tendencies, varied only within normal limits.

[4] The vitamin K concentrate used in these studies was kindly supplied by Dr. H. J. Almquist, whom we wish to thank. The material was injected intraperitoneally in four cases and fed with bile salts in the other two. The daily dose administered varied from 5 to 15 mgm. of the concentrate.

The other six animals which exhibited low blood prothrombin values (30–50 per cent) and which did not receive any vitamin K concentrate were studied further. The prothrombin level was found to rise spontaneously at times to, or near, the normal level. The bleeding tendency disappeared in a parallel manner. Members of the group were continued on the diet for periods as long as 320 days. In these animals the bleeding tendency was no more pronounced or frequent than it had been after 6 to 8 weeks on the diet. The proportion of animals exhibiting a low blood prothrombin associated with a hemorrhagic tendency was not greater in the third than it was in the first generation animals.

Thus, while it has been possible to produce by dietary means a bleeding tendency associated with a low blood prothrombin value in a fraction of the animals used, and to cure this deficiency by vitamin K concentrate, it has not been possible to increase the frequency or severity of this syndrome by depletion periods extending through three generations. The spontaneous recovery of some of the animals tended to indicate that the rats were receiving the factor in spite of the fact that the diets were repeatedly checked and found to be very low in vitamin K.

Sixty-five of the 77 animals used in these studies maintained blood prothrombin values near normal (70–100 per cent) even after extended periods of depletion. Some of these animals exhibited an increased bleeding tendency at one time or another. Attempts to correlate this condition with a lowered blood prothrombin level were unsuccessful. A few days later the abnormal tendency to bleed would disappear spontaneously. These rats grew, reproduced, and raised the young in a normal manner.

The possibility of bacterial synthesis of vitamin K in the intestinal tract was next investigated. An ether extract of feces collected from animals which had been maintained on the vitamin K-free diet for extended periods of time, when added to the basal diet and fed to young chicks, completely protected the chick from vitamin K deficiency (see table 1, group I). Rats raised on the deficient diet do therefore excrete significant amounts of vitamin K in their feces. Almquist (12) (13) has advanced evidence to indicate that bacteria inhabiting the intestinal tract may synthesize vitamin K. Chicks raised on the vitamin K-deficient diet, but allowed access to their feces will not develop the disease (12). Our results likewise indicate that rats raised on vitamin K-deficient diets continue to excrete significant amounts of the K-factor. It seems probable, therefore, that our failure to produce vitamin K-deficiency symptoms consistently in normal rats raised on the vitamin K-free diet is due to a bacterial synthesis of the factor in the intestinal tract of the animal.

This hypothesis was further tested. Thirty-two of the 77 animals which

had been maintained on the vitamin K-free diet for extended periods of time were jaundiced by double ligation and section of the common bile duct. The animals exhibited no increased hemorrhagic tendency at the time of operation, yet 2 to 9 days later they exhibited all the signs of the deficiency and would bleed so extensively from trivial injuries (see table 2, group IV) as to endanger the life of the animal. The bleeding times of some of the animals exceeded 36 hours; 9 bled to death as a result of clipping off the end of the tail with scissors. In many cases the end of the tail had to be ligated to prevent further loss of blood. The blood clotting time was practically normal until the bleeding tendency was well developed. The blood prothrombin level fell rapidly after the operation and was below 25 per cent of normal in most of the cases at the time the bleeding tendency appeared. Forty-seven animals which had been raised on a

TABLE 1

GROUP NUMBER*	EXTRACT PREPARED FROM	INITIAL DEPLETION	NUMBER OF CHICKS	NUMBER OF DAYS TREATED	FINAL CLOTTING TIME	ANIMALS EXHIBITING HEMORRHAGIC SYMPTOMS DURING TEST	PROTECTION
		days			*minutes*	*per cent*	
1	Normal† rat feces	18	5	13	2.5–8.0	0	Good
2	Jaundiced rat feces	16	6	10	2.0–11.0	0	Good
3	Bile fistula rat feces	18	6	10	3.5–9.0	0	Good
4	None	14	7	None	All over 30 minutes	100	None

* Only chicks having clotting times greater than 30 minutes at the end of the depletion period were used in these studies.

† These animals had been raised for periods of time ranging from 30 to 180 days on vitamin K-free diets at the time collections were made. Feces were collected in dilute HCl and extracted with ether and alcohol.

complete stock diet developed the bleeding tendency only 3 to 6 weeks following the operation (see table 2, group III). About one-half of the operated animals exhibited hemorrhagic tendencies.

Bile fistulas into the colon were established in 12 of the animals raised on the vitamin K-free diet (see table 2, group II). These animals exhibited no increased hemorrhagic tendency at the time the operation was performed. Within a week after the operation the bleeding times of all these animals were well over an hour. In some cases the bleeding continued for periods as long as 36 hours after clipping the end of the tail. Three of the bile fistula animals bled to death in this way. In 5 cases the tail was ligated at the end of the third hour to prevent further loss of blood. The blood prothrombin values of the members of this group were low (5 to 35 per cent). The administration of vitamin K concentrate prevented

or corrected the condition in the 7 cases treated. Animals raised on the stock diet and transferred to the vitamin K-free ration at the time the bile fistulas were established develop the deficiency much more slowly (see table 2, group I).

The possible rôle of infection or liver damage in the production of the syndrome was next investigated. In eight rats raised on the vitamin K-free diet the bile duct was connected to the vas deferens. In two of these cases the fistula remained open for a period of 14 days or more and in each case low prothrombin values, together with a marked bleeding tendency, developed. In these cases, as well as the obstructive jaundice cases, the

TABLE 2

GROUP NUMBER	OPERATION	DIET	NUMBER OF ANIMALS IN GROUP	AVERAGE NUMBER OF DAYS ON VITAMIN K-FREE DIET BEFORE OPERATION	AVERAGE PROTHROMBIN VALUE BEFORE OPERATION (PER CENT OF NORMAL)	DAYS AFTER OPERATION PRIOR TO APPEARANCE OF HEMORRHAGIC TENDENCY	ANIMALS EXHIBITING MARKED HEMORRHAGIC TENDENCY
							per cent
I	Bile fistula	Stock	45	None	100	13–60 ave. 24	80
II	Bile fistula	Vitamin K-free	22*	86	85	3–8 ave. 5	100
III	Jaundiced	Stock	47	None	100	18–42 ave. 26	50
IV	Jaundiced	Vitamin K-free	32†	119	80	2–9 ave. 4	94
V	None‡	Vitamin K-free	29			30–181+	25

* Ten of these animals died soon after the operation and are not included in the calculations.

† Fifteen of the animals had been on vitamin K-free diet 60 days, 13 for 180 days, and 4 for 137 days at the time the operation was performed. The results in all cases were similar.

‡ Days in this group refer to day on diet. Only 7 of this group showed a marked bleeding tendency although some of the animals were on the diet 320 days.

possibility of ascending infection via the bile duct was eliminated. That infection or liver damage is not the true cause of the syndrome is also indicated by the following: 1, at autopsy the livers of most of the bile fistula animals appear normal; 2, white cell counts made on the blood of some of our operated animals were usually within the normal range; 3, the condition in both bile fistula and jaundiced animals can be cured or prevented by the parenteral administration of small amounts of vitamin K concentrates; 4, the response to treatment can be carried out repeatedly; 5, relief of the icteric condition and subsequent repair of liver damage in the jaundiced groups is not essential to the action of vitamin K concentrates

in the control of the bleeding tendency; 6, it has been possible to maintain bile fistula rats in a state of health and control the hemorrhagic tendency for periods as long as 2 years by adequate weekly treatment with bile supplements; 7, we have had several animals with extensive subhepatic abscesses which have failed to respond to the administration of large doses of vitamin K and have died from hemorrhage during treatment; 8, blood fibrinogen was found to be within the normal range in the bile fistula rats and increased in the jaundiced animals; 9, animals raised on our stock diet do not develop hemorrhagic tendencies until 3 to 5 weeks after the drainage of bile in the intestine has been altered, whereas those initially depleted of a portion of their vitamin K reserves will develop the syndrome within a much shorter period (see table 2).

Ether extracts of feces collected from bile fistula, jaundiced, or normal rats maintained for extended periods of time on vitamin K-free diets, completely protect chicks raised on a vitamin K-free diet. (See table 1.) Thus the bile fistula and jaundiced animals are actually excreting significant amounts of vitamin K even at the time they are exhibiting a bleeding tendency which can be cured by adequate treatment with vitamin K concentrate. Previous work has indicated that the feeding of beef or sheep gall-bladder bile will control the hemorrhagic tendency even in bile fistula rats maintained on a vitamin K-free diet. This suggests the bile as a possible channel for the excretion of vitamin K. Feeding tests carried out on chicks indicate that beef and sheep gall-bladder bile contains but small quantities of vitamin K although good protection from gizzard erosion was obtained. Almquist has reported similar results (14).

The dramatic action of vitamin K concentrates in the cure or prevention of the hemorrhagic syndrome in the bile fistula or jaundiced rat indicates the necessity of vitamin K for this animal. The difficulty encountered in the consistent production of the deficiency in the normal animal can be explained by the synthesis of minimum amounts of the factor in the intestinal tract. In animals in which the biliary drainage into the intestine has been altered the intestinal absorption of this factor falls below the minimal requirements of the animal and the symptoms develop. The rapid and intense production of the syndrome after operation, in animals raised for extended periods of time on the deficient diets indicates a depletion of body stores of the essential factor. The relief of the deficiency by vitamin K concentrates indicates that a vitamin K-like substance is the factor involved. Bile salt feeding to bile fistula rats maintained on vitamin K-free diet does not give rise to an amelioration of the hemorrhagic tendency within a six day period, whereas the administration of vitamin K concentrates with the bile salts leads to a rapid recovery. This indicates that the amount of vitamin K available in the intestinal tract of

animals on the deficient diet is small. These data are consistent with the fact that we were able to produce deficiency symptoms in a small percentage of animals by dietary means alone. It also explains the spontaneous relief of symptoms which occurred in some of our animals. That the liver is not a major store house of vitamin K in the rat's body is indicated by the fact that supplementing the vitamin K-free basal diet fed to chicks with 10 per cent dried rats' liver does not protect the chick from hemorrhage.

SUMMARY

Young rats have been raised for extended periods of time on a vitamin K-free diet. Twelve of the 77 animals developed hemorrhagic tendencies associated with a low blood prothrombin. The deficiency was corrected by vitamin K concentrates. In the majority of the animals the deficiency could not be produced by dietary means alone. However, these animals rapidly developed the disease when drainage of bile into the intestinal tract was altered. The disease was cured by the administration of vitamin K concentrates. Bile fistula, jaundiced, or normal rats raised for extended periods of time on a vitamin K-free diet still excrete significant amounts of vitamin K. The part played by liver damage or infection is discussed. Bile is a poor source of vitamin K. Vitamin K is not stored in appreciable quantities in the rat's liver. Further evidence is afforded indicating that bile is essential to the intestinal absorption of vitamin K in the rat.

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THE ELASTIC PROPERTIES OF THE BEATING TORTOISE VENTRICLE WITH PARTICULAR REFERENCE TO HYSTERESIS

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Since the early work of Blix (1892), a great deal of attention has been paid to the visco-elastic properties of skeletal muscle. Quantitative investigations of the viscosity and elasticity of skeletal muscle at rest and during contraction have been carried out by Gasser and Hill (1924), Levin and Wyman (1927), Lindhard and Möller (1928), Sulzer (1928), and Bouckaert, Capellen and de Blende (1930).

Despite the great interest in the visco-elastic properties of skeletal muscle, surprisingly little work has been done on the elastic characteristics of cardiac muscle. More attention has been paid to the physiological property of its so-called tonus. Starling (1922) has given a purely physiological significance to tonus as a measure of the work capacity of the heart. Wiggers (1934) and Meek (1927) have defined tonus as "a sustained partial contraction, independent of systolic contractions, by virtue of which the muscle fibers resist stretching more than they would on account of their inherent elastic properties alone." A more universal definition of cardiac tonus has been proposed by Henderson (1923), who defines tonus as the degree of resistance to deformation. Because of the great confusion in the definition of the term, it has seemed to many that it would be desirable to discard the term tonus altogether in speaking of the heart. When using tonus in the sense of Henderson, one could speak of the elasticity of the myocardium. As Wiggers uses the term, tonus implies an increase in elasticity over the inherent elasticity of the heart muscle. In the case of the ventricle it has been impossible to determine whether such a sustained partial contraction ever really occurs, therefore we have chosen to consider only physical elasticity in the present state of our knowledge.

A study of the elastic properties of cardiac muscle under various conditions is important because the elasticity of the myocardium is a significant factor in the determination of the diastolic fiber length at any given filling pressure. The diastolic fiber length is of importance because it determines the magnitude of energy liberation in contraction (Starling and Visscher, 1927).

Studies of elasticity have been made by Frank (1895), Bruns (1914),

Sulzer (1932) and others on the frog heart, and Kozawa (1915) on the tortoise ventricle. The influence of changes in arterial pressure, heart rate, filling pressure, and temperature upon the diastolic volume of the tortoise ventricle has been studied by Moldavsky and Visscher (1933).

Because of the presence of the two separate ventricles, an investigation of the elastic properties of the mammalian heart is a much more difficult problem. The most significant work on the elasticity of the mammalian heart is the recent study of Johnson and Katz (1937).

In the present investigation, an attempt has been made to study the elastic properties of the isolated tortoise ventricle under various mechanical conditions. This work was suggested in part by the observation of Moldavsky and Visscher (1937), that after working for a time against large aortic pressures the tortoise ventricle requires more contractions for complete emptying against lower pressures than it does after working against lighter loads. This finding seemed to suggest changes in elasticity as a result of alterations in working conditions. Changes in elasticity have been correlated with changes in the external work of the ventricle in spontaneous isotonic contraction. The familiar pressure-volume relationship has been determined as a quantity dependent upon the modulus of elasticity (stress / strain).

Making certain assumptions regarding the shape of the ventricle at various pressures one can calculate the stretch modulus in absolute units. Upon so doing we have found that the value varies continuously during stretching, and no individual condition seems to us to be of any more significance than another as a basis for comparison. Consequently, we have presented the simpler pressure-volume curves for comparison, since they supply all the information which appears to us to be significant.

EXPERIMENTAL METHOD. The isolated ventricle of the tortoise was found to be suitable for this study of the elastic properties of cardiac muscle. Small hearts were more satisfactory than large ones because, in the latter, the thickness of the wall made diffusion inadequate as a means of oxygenation. The size of the heart is especially important because anoxia is one of the factors which modifies the elasticity of the ventricle.

After the beating heart was carefully exposed, a large bore cannula was inserted into the ventricle through a slit in the auricle and held firmly by a ligature at the atrio-ventricular junction. Then the aorta was ligated, the ventricle isolated, and the cannula was placed into the perfusion system.

The apparatus used is pictured in figure 1. The venous reservoir was filled with perfusion fluid which was prepared by diluting defibrinated turtle blood five times with Ringer's fluid. The whole perfusion system was kept full of fluid, care being taken to remove all air from the entire

system. By using the 1 cc. syringe, graduated in hundredths, and by proper adjustment of stopcocks *A* and *B*, exact quantities of fluid could be introduced or removed from the system at will. Changes in pressure were read off directly from the pressure-recorder, which consisted of a vertical glass tube of even bore, calibrated in millimeters. The pressure has been recorded as centimeters of water, although the perfusion fluid was obviously of a higher specific gravity than water. The error introduced by this discrepancy is constant and small. Measurement of ven-

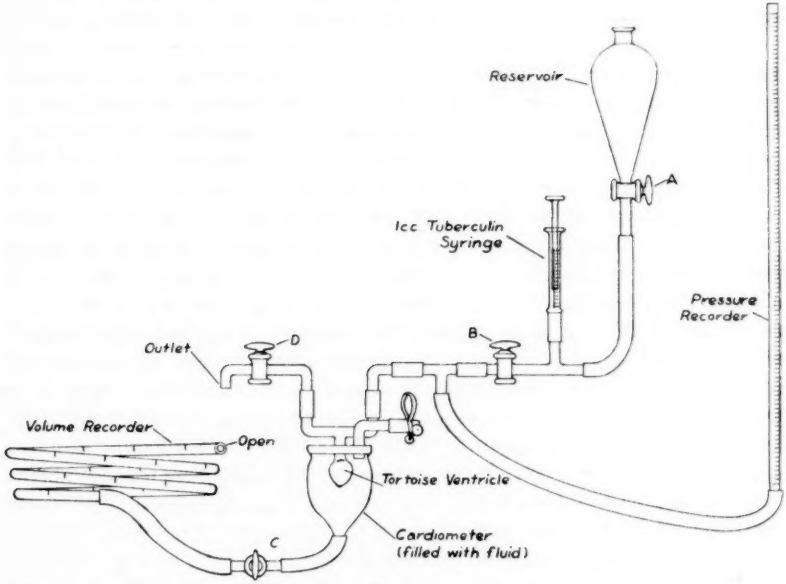

Fig. 1. Diagram of the apparatus. The pressure recorder is a straight glass tube with an internal diameter of 4.5 mm., calibrated in millimeters. The cannula to which the ventricle is attached has an internal diameter of 6 mm. The volume recorder is a concentric glass tube accurately calibrated in 0.5 cc. and fixed at the level of the ventricle. Not drawn to scale.

tricular volume was accomplished by means of the cardiometer which was completely filled with fluid, changes in volume being recorded by displacement of fluid from the cardiometer into the calibrated horizontal volume-recorder. The fluid in the cardiometer system was more dilute in protein than that in the perfusion system, consisting of turtle blood diluted ten to twenty times with Ringer's fluid.

This very simple method was finally adopted after testing several automatic recording systems. Membrane manometers and a mercury

manometer were tried for pressure recording but were abandoned because one of the objects of the experiments was to have normal isotonic contractions and it was found that permitting the ventricle to pump directly into the manometer tube provided the best arrangement. Mechanical recording was found to be superfluous with such a slow organ as the tortoise ventricle. Likewise in the case of the cardiometer the concentric circular horizontal tube with measurement of the position of the end of the column of fluid in it was found to be more satisfactory than either a piston recorder, a spirometer or a tambour, particularly because it was desirable that no pressure changes occur on the outside of the ventricle.

Most of the experiments were performed with the apparatus in air at room temperature, 23 to 25°C., but in several experiments the apparatus was submerged in a water bath at 20°C. After waiting for equilibration of the perfusion fluid with the environmental temperature, the heart was perfused continuously for several minutes with perfusion fluid that had been adequately aerated. Then the ventricle was emptied at a moderate negative pressure, 5 cm. H_2O, until no further decrease in volume could be obtained. This was considered the zero volume. It must be noted that the zero volume is not reached as soon as a negative pressure is applied. Especially after distention, it takes some time for the ventricle to empty itself completely. Then the ventricle was filled stepwise and records were made of the lowest pressure and the greatest volume reached in diastole, and the highest pressure and smallest volume reached in systole with each increment of fluid in the system. Several readings of systolic and diastolic pressure and volume were made after each addition of fluid. When the ventricle was somewhat distended, the procedure was reversed and 1 cc. of fluid was withdrawn at a time, thus gradually emptying the ventricle; records were made of pressures and volumes after each withdrawal of fluid. Such a series of observations took several minutes.

Following this series of observations, the heart was perfused for several minutes with fresh aerated fluid. Then the zero volume was again determined and another series of observations made. Usually the measured zero volume was approximately the same as at the previous observation, indicating that only a small amount of filtration of fluid through the ventricular walls had occurred. Five to ten such series of observations were made in the course of each experiment. Care was taken to measure the zero volume at the beginning and at the termination of each series of observations because of this factor of filtration of fluid through the walls of the ventricle into the cardiometer system. Throughout an experiment, the ventricle continued to beat spontaneously.

The errors involved in the measurement of the pressure and volume were small. Momentum of fluid in the pressure and volume recorders as a result of contraction of the ventricle perhaps introduced a small error

in systolic measurement. An error was introduced in the diastolic volume readings as noted above by the increase in fluid in the cardiometer system resulting from filtration of fluid through the ventricle. This filtration was especially evident at high filling pressures so that an increase of apparent zero volume of 0.05 to 0.10 cc. in the course of a series of observations was quite common. It must be noted that the heart was beating spontaneously and the heart rate was not controlled. Although the heart rate was greater at greater diastolic volume, the range of variation of the heart rate was probably small enough so that no significant difference in filling of the ventricle and consequently in output could result from changes in heart rate.

The external work of the ventricle in isotonic contraction was calculated by measuring the area under the curve of pulse pressure plotted against volume output per beat. The calculation was made by the formula:

$$W = \frac{1 + 2 + 3 \cdots + PP}{VF}$$

where W = work in gm. cm.
PP = pulse pressure in cm. H_2O
VF = volume factor of pressure manometer (cm./cc.)

The kinetic factor was neglected in calculating the work. In this calculation, the error is less at the higher pulse pressures. In comparing the work at the same diastolic volume under different conditions, however, the error remains rather constant through most of the range of pulse pressure changes observed. Thus the comparisons of work in the same heart under different conditions are definitely significant, in spite of the fact that the work as calculated is admittedly always less than the true value.

Results. As is well known, when the ventricle is gradually filled with fluid, the diastolic volume at first increases with relatively little increase in diastolic pressure. Then as filling continues, the pressure rises more and more with each increase in volume, so that when the ventricle is much distended, further addition of fluid causes the pressure to increase more sharply than the volume. The normal diastolic pressure-volume curve of the isolated ventricle is thus hyperbolic as shown in figure 2.

The pressure-volume curve for the height of isotonic contraction in the normal isolated tortoise ventricle is a curve which rises sharply and is concave downwards (fig. 2). Retention of much fluid in the ventricle at the end of systole takes place only at higher filling pressures. At the higher filling pressures, the pulse pressure begins to decrease, indicating that the diastolic volume is beyond the optimum for ejection.

If the ventricle is filled progressively, emptied abruptly and then filled again, 1 cc. at a time, with aerated blood, the diastolic pressure-volume

curves show that the elasticity is less during the second filling (fig. 3). The record of the third filling demonstrates a further decrease in diastolic elasticity. In other words, as the experiment proceeds, there is a progressive decrease of the diastolic elasticity of the ventricle, despite the fact that the ventricle is not greatly distended and is perfused with aerated blood between fillings. As a rule, the diastolic elasticity decreases steadily for four or five fillings and then remains approximately constant at the lower level in subsequent fillings.

Along with this progressive decrease in diastolic elasticity, there is a decrease in the external work of the ventricle (fig. 3), Since there is

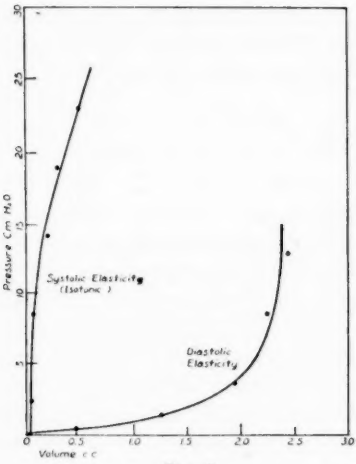

Fig. 2

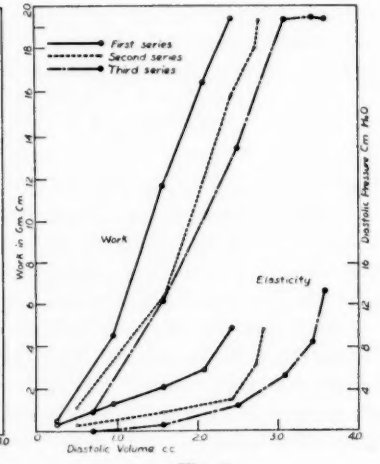

Fig. 3

Fig. 2. Characteristic pressure-volume curves of the fresh tortoise ventricle in diastole and in systole.

Fig. 3. The effects of successive fillings on elasticity and on external work of the tortoise ventricle. Note the progressive decline of the diastolic pressure-volume curves correlated with the progressive decrease of the external work of the ventricle.

evidence that the energy utilization at any given diastolic volume of the tortoise ventricle is constant (Decherd and Visscher, 1933), a decrease in external work at the same diastolic volume indicates a decrease in mechanical efficiency. A decrease of efficiency in progressive failure is characteristic not only of the isolated ventricle of the tortoise (Decherd and Visscher, 1934), but also of the isolated mammalian heart in the heart-lung preparation (Peters and Visscher, 1936), and of the isolated mammalian heart perfused with blood aerated by means of an oxygenator (Moe and Visscher, 1939). Since, in our experiments, the progressive decrease of elasticity always accompanies the progressive decrease in

efficiency of the ventricle, it seems probable that a decrease of elasticity is characteristic of failure in the tortoise heart. Furthermore, Johnson and Katz (1937) have recently demonstrated a decrease in the elasticity of the myocardium in spontaneous heart failure in the dog. It is thus probable that one factor in the dilatation of the failing heart is the decrease of elasticity of the myocardium. This decrease is definitely advantageous to the failing heart, since the diastolic pressure does not rise as high in failure as it otherwise would.

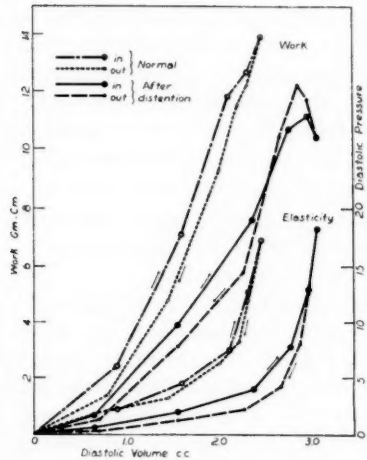

Fig. 4

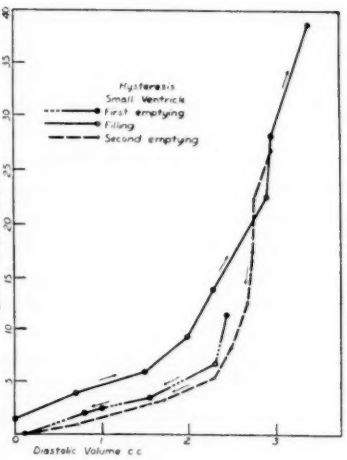

Fig. 5

Fig. 4. The effect of distention on the elasticity and on the external work of the tortoise ventricle. Ascending arrow indicates filling and descending arrow indicates emptying of the ventricle. Note the elastic hysteresis and the correlated changes in external work.

Fig. 5. Curves showing elastic hysteresis of the ventricle. Ascending arrow indicates filling and descending arrow indicates emptying. Note that the emptying curve is further to the right whether it precedes or follows the filling curve.

Over-distention of the ventricle for a period of five to ten minutes at a very high filling pressure has been studied in several experiments. Distention of the ventricle for 10 minutes at a filling pressure of 45 cm. of water resulted in a marked and prolonged decrease of both diastolic elasticity and work (fig. 4). Following distention, readings were taken every fifteen minutes to determine the rate of recovery. It was found that recovery of diastolic elasticity was very slow under the conditions of the experiment, so that at one hour after distention, no appreciable increase in diastolic elasticity could be observed. This failure to recover normal

elasticity is a type of elastic fatigue. In one experiment in which the ventricle was distended and then kept at low pressure for several hours, some recovery of diastolic elasticity did occur, but elasticity was not restored to the normal level. Recovery of the external work, on the other hand, was much more rapid, so that a significant increase in work over that observed immediately following distention, was recorded one hour after distention.

When the ventricle is filled 1 cc. at a time and is then emptied progressively in the same manner, the diastolic pressure/volume curve for filling is at a higher level than that for emptying (fig. 4). In other words, there is a lag in the decrease of volume of the ventricle (or in the length of the muscle fibers) so that the elasticity is less on emptying the ventricle. This phenomenon, which is characteristic of most elastic materials, we have called "elastic hysteresis," after Bouckaert, Capellen and de Blende (1930). The elasticity of the ventricle is less during emptying than during filling whether the filling immediately precedes or follows the emptying of the ventricle (fig. 5). The temporary change in elasticity as a result of short periods of stretch is thus quite different from the change in prolonged distention, since the former is rapidly reversible and the latter is not. The degree of elastic hysteresis observed varied a good deal.

If the work curves for successive filling and emptying in the fresh tortoise ventricle are plotted, it is often observed that the work curve for filling is higher than that for emptying (fig. 4). On the other hand, after distention, in some hearts over the whole range of pressures and volumes, and in almost every instance at higher volumes, the work done at a given diastolic volume is greater on emptying than on filling the ventricle (fig. 4). We attribute this effect to the greater mechanical advantage under which the heart works at the lower diastolic pressures during emptying.

If the ventricle is deprived of oxygen by failure to flush through frequently with aerated perfusion fluid, there is a rapid and progressive decrease in elasticity. This is demonstrated by the successive curves for diastolic elasticity obtained in an experiment on a large, thick-walled tortoise ventricle (fig. 6). That this decrease in elasticity is due to lack of oxygen as well as fatigue is shown by the increase in elasticity observed following several minutes of perfusion with a well oxygenated fluid (fig. 6). We made no attempt to differentiate hypercapnia from anoxia in the production of this decreased elasticity.

Discussion. There is a striking similarity in the elastic properties of cardiac and of skeletal muscle. The pressure-volume curves of the fresh isolated ventricle in diastole and isotonic systole are almost identical with the tension-length curves of skeletal muscle at rest and in isotonic contraction. This similarity has been noted by Hewlett (1928) and by Sulzer (1932). Gasser and Hill (1924) demonstrated a marked increase in the modulus of elasticity of the sartorius muscle of the frog during tetanic contraction, as determined by the rate of damping of a steel spring.

Successive fillings of the tortoise ventricle lead to a progressive decrease in diastolic elasticity. In the same way, successive tension-length curves of skeletal muscle at rest show a progressive decrease of elasticity, as shown by Sulzer (1928). Furthermore, Levin and Wyman (1927) have observed that fatigue leads to a decrease of elasticity as well as an increase of viscosity of resting skeletal muscle. The phenomenon of elastic hysteresis has been observed repeatedly with skeletal muscle (Sulzer, 1928; Bouckaert, Capellen and de Blende, 1930), and forms the basis of the familiar laboratory experiment on the extensibility of muscle. Sulzer (1932) has noted the similarity of hysteresis curves for skeletal and cardiac muscle.

Working with cold-blooded hearts, Frank (1895) was the first to describe the characteristic pressure-volume curves of diastole and systole. Despite the claim of Bohnenkamp and Ernst (1926) that the elasticity curve

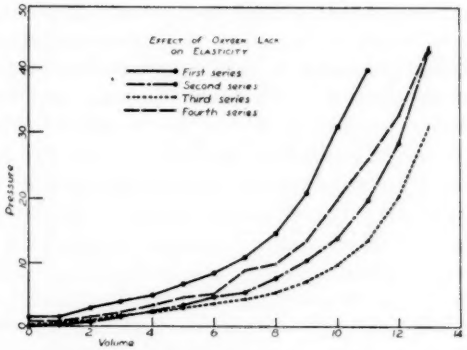

Fig. 6. Effect of oxygen lack on the elasticity of a large tortoise ventricle. No oxygen was added to the perfusion fluid during the first three series of observations. Preceding the fourth series, the ventricle was perfused with well-aerated fluid for several minutes.

for the resting heart is a straight line, most investigators, including Bruns (1914), Kozawa (1915), Kuraya (1924), Müller (1930), Sulzer (1932), Katz (1932) and others have confirmed Frank's findings.

Successive fillings of the ventricle and fatigue lead to a progressive decrease in diastolic elasticity as well as in the curve of isotonic maxima, according to Sulzer (1932) and Katz (1932). Recently, Johnson and Katz (1937) have demonstrated that a decrease of diastolic elasticity also occurs in the mammalian heart in spontaneous failure.

Similar decreases of diastolic elasticity and of systolic elasticity have been described following overdistention of the frog ventricle by Bruns (1914) and Sulzer (1932). Brams and Katz (1931) have studied the effects of overdistention of the mammalian heart in situ by massive transfusion into the veins. They found that the diastolic pressure fell signifi-

cantly on release of the overdistended heart by venesection, while the diastolic volume changed very little as a rule. This indicates a decreased diastolic elasticity following overdistention. They also observed that the output per beat was usually reduced following overdistention. Recovery of strength of beat and of diastolic elasticity after overdistention has been reported by Bruns (1914) in the frog ventricle.

Hysteresis in the cold-blooded heart has been observed by a number of workers, including Kuraya (1924), Sulzer (1932) and Moldavsky and Visscher (1937). No previous investigators have correlated work and elasticity in hysteresis. The phenomenon of hysteresis may play a significant rôle in the dynamics of the heart in conditions of clinical importance, such as after venesection.

The consensus of opinion seems to be that asphyxia of the myocardium leads to cardiac dilatation partly as a result of a decrease in elasticity. Andrus (1919) has shown that a small decrease of pH decreases elasticity. Kozawa (1915) maintains, on the other hand, that perfusion of a tortoise ventricle with fluid containing a high concentration of CO_2 causes an increase of diastolic elasticity. However, Gremels and Starling (1926) have observed that a decrease of pH resulted in dilatation of the isolated mammalian heart under controlled conditions of the circulation. There seems to be some difference of opinion concerning the rôle of hypercapnia and anoxia in producing the decreased diastolic elasticity observed in asphyxia. Wiggers (1929) found no change in diastolic volume as a result of hypercapnia alone, in dogs with heart rate, venous return and arterial resistance controlled. Strughold (1930) found a decrease of elasticity in the dog heart, as a result of moderate anoxemia. On the other hand, Patterson (1914) found that CO_2 administration increased heart volume in the heart-lung preparation, and Gremels and Starling (1926) found that a decreased pH was a more potent factor in producing cardiac dilatation than anoxemia.

The contribution of the present series of experiments is, first, in showing that short periods of application of high pressure result in a reversible decrease in elasticity (the hysteresis effect) while longer periods of pressure application lead to more permanent lowering of elasticity (elastic fatigue); second, that the decreased elasticity in the state of anoxia is reversible; third, that in this preparation the work capacity decreases when elasticity falls but the former may increase more than the elasticity in the recovery from prolonged stretching; and fourth, that at relatively high filling pressures the work capacity is greater when the elasticity is low.

SUMMARY AND CONCLUSIONS

1. The diastolic elasticity curves of the isolated ventricle have been studied in the tortoise heart under various mechanical conditions.

2. The elasticity of the ventricle decreases progressively in successive fillings. The decrease in elasticity is correlated with a decrease in the external work of the ventricle, indicating that a progressive decrease in elasticity is associated with spontaneous failure.

3. Overdistention of the ventricle for a short time is followed by a marked decrease in elasticity and in external work. The work gradually increases in the course of an hour, but there is relatively little increase in the elasticity during that period.

4. The elasticity is greater on filling the ventricle than on emptying it. This phenomenon has been called "elastic hysteresis." In the fresh heart, the external work is also greater on filling the ventricle than on emptying. But after overdistention, the work is greater on emptying than on filling the ventricle, especially at the higher diastolic volumes.

5. A significant decrease in elasticity can be produced by relative asphyxia of the ventricle. After perfusion with oxygenated fluid, the elasticity is observed to recover.

6. The similarity of the elastic properties of skeletal and cardiac muscle is discussed.

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DECUSSATIONS OF SYMPATHETIC EFFERENT PATHWAYS FROM THE HYPOTHALAMUS[1]

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It has been shown that in the region of the hypothalamus, there exists a center or centers for sympathetic functions (Ranson and Magoun, 1939), and that the impulses from one side of the hypothalamus activate effectors bilaterally. However, knowledge regarding the level of cross connections is still fragmentary and conflicting.

Karplus and Kreidl (1912) found that the descending pathway from one side of the hypothalamus reaches both cervical sympathetics by a cross connection at the level of the upper thoracic cord. Bumke and Foerster (1936) stated that the sympathetic fibers which influence piloerection, bladder and intestinal responses, and temperature regulation decussate partially in the pons and medulla. Magoun, Ranson and Hetherington (1938) found that a small portion of the descending vasomotor impulses from the hypothalamus are crossed above the first cervical segment of the spinal cord. Since a generalized sympathetic manifestation is usually observed during intense stimulation (Cannon, 1929), and since the impulses concerned in all types of sympathetic activity are known to descend in the anterolateral column of the spinal cord (Bumke and Foerster, 1936) it would not be surprising if the various physiological fiber types decussate at about the same level. The present study was undertaken to investigate the level or levels of fiber crossing.

METHODS. In 8 cats, the sympathetic chain on one side was removed aseptically from the stellate ganglion down according to the technique described by Cannon, Newton, Bright, Menkin and Moore (1929). The postoperative course was uneventful. All animals appeared healthy and behaved in no way different from what Cannon et al. (1929) have reported.

Five to 7 weeks later, stimulation experiments were carried out under light nembutal anesthesia (25 mgm. per kilo, intravenously). The cervical sympathetic nerve on the sympathectomized side and both vagi were carefully isolated. The blood pressure was recorded from the right carotid artery by means of a mercury manometer.

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The animal was placed in a prone position, the skull was trephined and cervical laminectomy at the level of C_{5-7} was performed. In one animal, the oculomotor nerve of the normal side was severed intracranially. A Horsley-Clarke stereotaxic instrument was then adjusted to the cat's head and fastened to a stand with a 25 degree ventral flexion of the head.

For recording the movements of the nictitating membranes, fine silk threads were attached to the free borders and led over pulleys to two similar isotonic levers, each carrying a 5 gram load and giving approximately ten-fold amplification. In each case, the cervical sympathetic nerve on the sympathectomized side was cut and its peripheral cut-end stimulated for detecting undegenerated fibers.

Bipolar nichrome wire electrodes with tips separated by 0.5 mm. were inserted by means of the Horsley-Clarke instrument. The stimulating current was provided by a Harvard inductorium with its primary connected to a single dry cell of 1.5 volt and secondary placed at 9.0 cm. The hypothalamus on the sympathectomized side was usually explored first. After a few satisfactory responses on blood pressure and the nictitating membranes were obtained, the electrode was shifted to the opposite side and the responsive region located. The electrode was left at the latter point while a hemisection of the cord was performed at the level of C_{5-6} or C_{6-7} on the normal side. Stimulation was then repeated on both sides. In 2 of these cats, the hemisection was carried out aseptically 1 to 3 weeks prior to the stimulation experiment. Before terminating each experiment both vagi were cut, the floor of the fourth ventricle was exposed and the myelencephalic center, as described by Chen et al. (1936), was stimulated with bipolar silver wire electrodes.

At the conclusion of the experiment, the animal was perfused with 10 per cent formalin. The completeness of the unilateral sympathectomy was checked, and the brain was taken out and the stimulated region examined grossly for the symmetry of the punctures in the hypothalamus. The level of the hemisection was determined and the cord at the level of the section was removed, embedded in celloidin, cut serially and stained by the Weil method. The extent of the hemisection was subsequently examined and recorded with a microprojector.

RESULTS. *Hypothalamic responses after unilateral sympathectomy.* Stimulation on the normal side of the anterior hypothalamus (fig. 1a) usually gave a prompt rise of blood pressure and a contraction of the normal ipsilateral nictitating membrane, *NN*. The contralateral sympathectomized membrane, *DN*, showed a belated, slow contracture, lasting for a few minutes (fig. 1a). The responses following hypothalamic stimulation of the sympathectomized side (fig. 1b) were usually similar but of less magnitude; not infrequently, however, responses of the same size were obtained on both sides (table 1).

Hypothalamic responses after unilateral sympathectomy and hemisection of the contralateral cord. After the hemisection of the cervical cord of the normal side at the level of C_{5-6} or C_{6-7}, the vasomotor responses were reduced, especially if the section was at the lower level (cats 1, 2 and 3); and

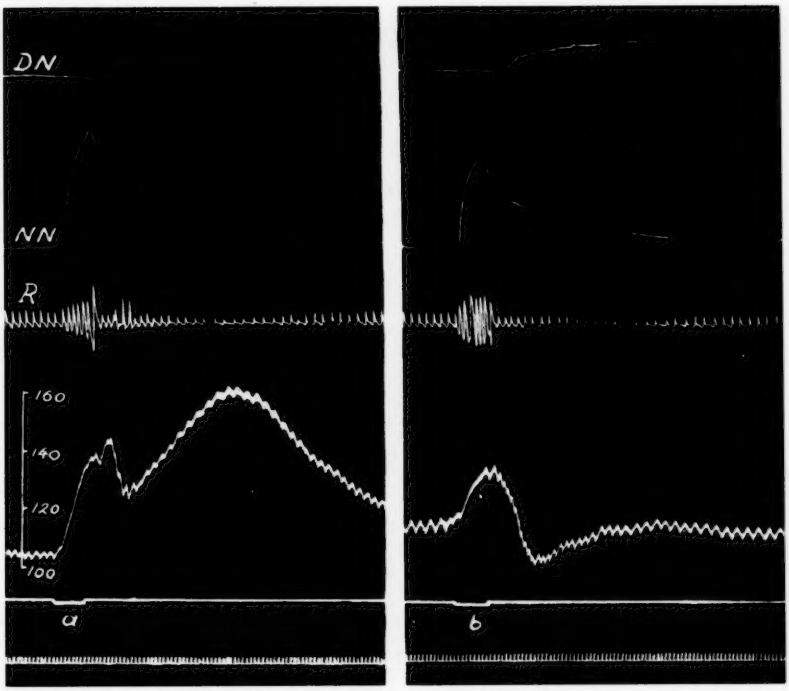

Fig. 1. Effects of stimulation of the anterior hypothalamus in a unilaterally sympathectomized cat (cat 1) on blood pressure, nictitating membrane (normal, *NN*; and sympathectomized, *DN*) and respiration (*R*). Time in seconds.

a. Responses following stimulation of the hypothalamus of the normal side.

b. Similar but smaller responses following stimulation of the sympathectomized side.

the reduction was much greater following hypothalamic stimulation of the hemisected side. Nevertheless, a positive response was always obtainable.

The influence of such a hemisection on the contraction of the normal nictitating membrane following stimulation of the hypothalamus on the sympathectomized side was very slight. The response to stimulation of the hemisected side, on the other hand, was definitely impaired (table 1). The denervated nictitating membrane in two cases failed to respond to

central stimulation on either side after hemisection of the cervical cord; and in another the response was altered in very much the same way as that of the normal nictitating membrane.

Hypothalamic responses after unilateral sympathectomy and chronic hemisection of the contralateral cord. In those cats with chronic cervical hemisections on the normal side at the level of C_{5-6}, responses could be obtained by stimulation of the hypothalamus on either side. Stimulation on the sympathectomized side gave a stronger pressor response and

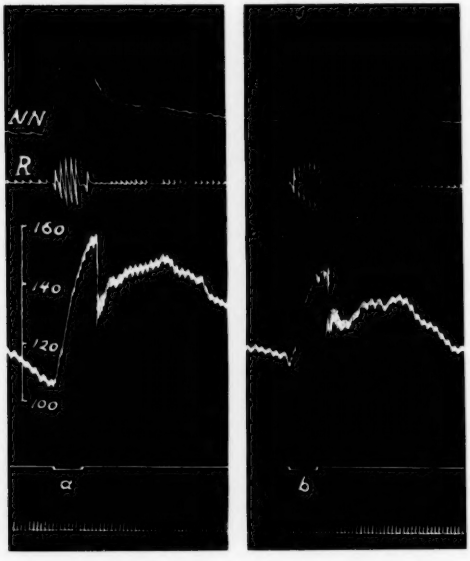

Fig. 2. Effects on contraction of the normal nictitating membrane (*NN*) and blood pressure of central stimulation in unilaterally sympathectomized cat (cat 6) with chronic cervical hemisection at C_{5-6} of the contralateral side.

a. Responses following stimulation of the sympathectomized side.

b. Responses following stimulation of the hemisected side. Both the rise of blood pressure and the contraction of nictitating membrane are smaller than a.

greater contractions of the nictitating membranes. On the hemisected side, the responses were smaller (fig. 2). However, in both the chronic and the acute animals the positive responses are significant.

Pupillary sphincters. Pupillary responses were not easily interpreted. Dilatation of pupils was encountered upon stimulating points over a wide area in the anterior hypothalamus. In most instances the dilatation on both sides was equal and independent of the state of its sympathetic innervation or of the side of stimulation. Occasionally a difference in the pupillary reaction was observed, but in those cases it was so irregular that

its significance could not be made out. In one experiment (cat 8) the oculomotor nerve was severed. The pupil so denervated was found to be so markedly dilated that further dilatation could not be detected on hypothalamic stimulation, although it still responded noticeably to stimulation of the cervical sympathetic nerve. In the two animals in which peripheral sympathectomy was performed on one side and chronic cervical hemisection on the other, a definite difference in the size of the pupils and the tonic state of the nictitating membranes was observed, which indicates that the structures on the hemisected side still have central connections.

TABLE 1

Vasomotor and nictitating membrane (normal and sympathectomized sides) responses following stimulation of the anterior hypothalamus in unilaterally sympathectomized cats, before and after hemisection of the cervical cord of the normal side

CAT NUMBER	LEVEL OF HEMISECTION	MAXIMUM RISE OF BLOOD PRESSURE IN MM. Hg				MAXIMUM CONTRACTION OF NORMAL NICTITATING MEMBRANE (× 10)				MAXIMUM CONTRACTION OF SYMPATHECTOMIZED NICTITATING MEMBRANE (× 10)				STIMULATION OF MYELENCEPHALIC CENTERS. MAX. B.P. RISE IN MM. Hg AFTER HEMISECTION	
		Before hemisection		After hemisection		Before hemisection		After hemisection		Before hemisection		After hemisection			
		Normal side	Sympathectomized side	Normal side	Sympathectomized side	Normal side	Sympathectomized side	Normal side	Sympathectomized side	Normal side	Sympathectomized side	Normal side	Sympathectomized side	Normal side	Sympathectomized side
1	C_{6-7}	42	20	0	5	22	16	17	14	6	4	0	0	—	—
2	C_{6-7}	16	16*	11	16									−5	50
3	C_{6-7}	28	24	10	20	8	7	3	7	4	2	0	0	4	30
4	C_{5-6}	34	30	28	30					7	7	4	6	0	100
5	C_{5-6}	30	30	10*	22	3	3	0	3					0	104
6†	C_{5-6}			34	50			12	18			4	4	0	80
7†	C_{5-6}			22	24							8	15	0	60

* Followed by a depressor reaction.

† Chronic hemisection of the cervical cord.

Myelencephalic vasomotor responses. Consistent results were obtained upon stimulating the myelencephalic sympathetic centers. Stimulation on the sympathectomized side yielded an elevation of blood pressure up to 100 mm. Hg; while stimulation on the hemisected side gave no significant alteration of blood pressure (table 1).

At autopsy, it was found that the unilateral sympathectomies were complete and points of stimulation in the hypothalamus were fairly symmetrical. The lesions in the cervical cord varied in extent but only the desired half was destroyed. The levels of hemisection are shown in the table.

Discussion. In bilaterally sympathectomized cats no prompt pressor

response is obtainable following central stimulation (unpublished data). Therefore, we may assume that any prompt change of the blood pressure and of tonus of the normal nictitating membrane due to hypothalamic stimulation on the side of the sympathectomy, shows the existence of cross connections to the intact sympathetic chain.

The efferent sympathetic pathway is said to be partially crossed in the pons and medulla (Bumke and Foerster, 1936), but this is denied by Spiller (1920). Magoun, Ranson and Hetherington (1938) have observed a residual pressor reaction upon stimulation of the ipsilateral hypothalamus after hemisection at the levels of the midbrain, pons and upper cervical cord; a greater reaction is obtained in the case of cervical section. They therefore concluded that the decussation of the vasomotor pathway occurred in part in the brain stem. In the chronic unilaterally sympathectomized cats there might have been some postoperative, functionally compensatory, cross connections. Nevertheless, stimulation of the hypothalamus still shows that the descending pathway is predominantly ipsilateral. The change following hypothalamic stimulation after hemisection of the cord at the level of C_{5-6} or C_{6-7} on the normal side is of interest; the reduction of responses on stimulating the sympathectomized side may be attributed to the severance of the descending pathway that has crossed above the hemisection, and the residual responses must be due to impulses transmitted through another cross connection in the spinal cord below the hemisection. The greater reduction obtained on stimulation of the hemisected side is probably explained by the destruction of a large uncrossed efferent tract, but the residual response certainly indicates that impulses having descended via the decussation in the brain stem have also a bilateral representation through cross connections in the spinal cord below C_7.

If the aforementioned interpretation includes the correct efferent pathway, it seems strange that following a hemisection, stimulation of the hypothalamus on the sympathectomized side revealed no alteration of pressor responses in cat 4 and no change of the normal nictitating membrane in cats 3 and 5. However, since in the normal cats the sympathetic response following hypothalamic stimulation very often becomes augmented after hemisection of the contralateral cord (Magoun, Ranson and Hetherington, 1938; Wang and Ranson, 1939), absence of any reduction in these cases may not mean that there is no decussation in the brain stem, but probably indicates that such efferent elements in these cats are relatively and functionally insignificant.

Magoun, Ranson and Hetherington (1937) have demonstrated that a sufficient amount of adrenin and sympathin is liberated upon stimulation of the hypothalamus to give a good response of the postganglionically denervated nictitating membrane. The reduction in the contracture of

the denervated membranes in our animals then may be interpreted as damage to the efferent pathway to the adrenal gland and other sympathetic end-organs by the section in the spinal cord. The data suggest that such an efferent pathway differs in no essential way from that of vasomotor and cervical sympathetics. The disappearance of such hormonal responses after hemisection of the cervical spinal cord in cats 1 and 3 may be explained by the increased threshold in the semi-shock condition. Positive responses in the cats with chronic cervical hemisection speak for this interpretation.

Karplus and Kreidl (1912) stimulated the hypothalamus of cats with bilateral hemisections at different levels of the cervical cord and found no evidence of crossing of impulses from the brain stem to the cervical sympathetics in the cervical segments. On several occasions they obtained cervical sympathetic reactions following stimulation of the hypothalamus of cortex (with strong current in the latter case) after the cervical cord had been hemisected on the ipsilateral side. These findings are, by and large, the same as our own observations reported here. But we fail to agree with Karplus and Kreidl that such findings can be accounted for by current spread or by assuming some connections between the hypothalamus on the two sides. The mere fact that stimulation of the midline between the two sides of the hypothalamus gives only a small response indicates that neither current spread nor direct hypothalamic connections is the likely explanation. We believe that there are diffuse cross connections in the brain stem as well as in the spinal cord below the cervical segments. Our results clearly demonstrate that hypothalamic efferent impulses being largely uncrossed, may descend to the contralateral side either in the brain stem or in the spinal cord below the cervical segments and a small portion of them may descend via both connections to reach the ipsilateral half of the spinal cord below the hemisection.

The descending pathway of the myelencephalic center has been located in the anterolateral column of the spinal cord (Sherrington, 1887). In the some location, the efferent pathway from the hypothalamus descends (Bumke and Foerster, 1936). Chen et al. (1937) found that high cervical chordotomy at C_2 would abolish the effect from stimulation of the ipsilateral sympathetic center in the medulla and thereby concluded that there is no crossing of the descending pathways in that region. In the present experiments, it is found that stimulation of the medullary center on the sympathectomized side remains effective and that it is ineffective on the hemisected side. This indicates that the hemisection is functionally complete and that the efferent pathway crosses below the section. However, from these experiments alone one cannot tell whether there is any crossing above the section, nor determine the percentage of the crossed and uncrossed elements. However, the slight difference in the pressor

response between the higher (C_{5-6}) and the lower (C_{6-7}) hemisection suggests that crossing has occurred in that vicinity. The elevation of blood pressure obtained from the medullary stimulation in these animals is much smaller than that in the normal (30–104 vs. 80–200 mm. Hg, Chen et al., 1936). It is therefore likely that the efferent tract is only partially crossed at this level.

SUMMARY

A method is described for the study of the level of decussations of the vasomotor efferent pathways from the hypothalamus: stimulation of the hypothalamus is made on unilaterally sympathectomized cats before and after a low cervical hemisection. With such a preparation it is possible to estimate the crossed and uncrossed components, not only of the vasomotor efferent but also of the cervical sympathetic and adrenal efferent pathways.

Hypothalamic impulses may descend to the spinal cord uncrossed, or cross in the brain stem or in the spinal cord below the cervical segments. It may also reach the ipsilateral half of the cord below a hemisection via connections both in the brain stem and spinal cord. It is assumed that the connections in the spinal cord are effected by a spinal commissural system.

The descending pathway from the myelencephalic sympathetic center decussates partially in the spinal cord below the cervical segments.

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THE EFFECT OF FASTING ON THE BLOOD SUGAR OF HEREDITARY DWARF MICE[1]

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When subjected to fasting, hypophysectomized animals of various species readily develop hypoglycemia which, if untreated, leads to death (1, 2). Thus starvation causes death in 18 to 24 hours in hypophysectomized rats (2) and usually within 12 hours in mice (3). These facts suggest that the anterior pituitary elaborates a hormone which contributes to the maintenance of a normal blood sugar level. Since hereditary dwarf mice lack the acidophil cells of the anterior lobe of the pituitary gland (4, 5, 6), a study of the blood sugar of these animals particularly during fasting should give information as to whether the hormone mentioned above is secreted by the acidophil rather than by the basophil or chromophobe cells. Such was the purpose of the experiments reported in this paper.

Kemp and Marx (5) consider that the relative weight of the pancreas of the dwarf mouse decreases with age, while that of the normal animal increases and that the number of islands is approximately inversely proportional to the size of the pancreas, i.e., the smaller the pancreas the more dense the distribution of islands. They also believe that growth hormone, in which the dwarf mice are deficient (4), stimulates the development of insular more than that of exocrine tissue. However, histological examination of the pancreas in our dwarfs which were more than a year old showed no obvious difference from normal controls in either exocrine or insular tissue.

The thyroid, thymus and adrenals of dwarf mice have been described as hypoplastic, indicating the persistence of an earlier developmental state than in the normal animal of the same age. Because of this, Kemp and Marx believe that the dwarfs may be deficient in one or more hormones (5). It has been pointed out that the dwarfs of both sexes may be partially or completely deficient in one of the gonadotropic hormones (F.S.H.) (7).

[1] This investigation was aided by a grant from the Proctor Fund of Harvard University.

PROCEDURE. Blood for analysis was obtained by stroking the animal's tail (warmed to promote vascular dilatation and blood flow) after cutting off the tip. The procedure used for the collection of blood and determination of sugar (micro method) was that of Folin (8). With a few exceptions, in the earlier part of the work 0.10 cc. of blood was collected in 10 cc. of precipitating fluid. The size of the sample taken was later decreased to 0.05 cc. to reduce the volume of blood lost by the animal; 5 cc. of precipitating fluid were then used. In a few instances, particularly in the fasting dwarf mice, only 0.02 to 0.04 cc. could be obtained although insofar as possible such small samples were avoided. In the most valuable series of determinations, those after a 96 hour fast, all samples were of 0.05 cc. amount with one exception and this consisted of 0.04 cc.

In order to control conditions as well as possible all animals were transferred to a room kept at 23° to 25°C. at least 48 hours before an experiment was begun and kept there throughout the experimental period. For 2 days prior to a test and throughout the non-fasting determinations all mice were given milk in addition to the usual diet of Purina dog chow. The blood for sugar determinations was drawn between 10 a.m. and 12 noon. During the bleeding the mice were held in a towel to minimize the effect of excitement upon the blood sugar level. The control animals used were albino mice from various sources and included some from strains C and C36 from the Roscoe B. Jackson Memorial Laboratory. In addition, black normal litter mates of the dwarf mice were used.

The first lot of dwarf mice used, obtained from Dr. E. C. MacDowell of the Carnegie Institution, were of the original silver strain in which the gene was discovered. There were 2 males and 6 females born June 15 to July 24, 1936. All others were bred in this laboratory from a heterozygous stock, which carried other known genes, e.g., those for waltzing, flexed-tail and recessive spotting, but only 2 of the dwarfs used in these experiments showed any abnormality other than dwarfism. These two were excessively fat.

RESULTS. In table 1 are shown the average blood sugar values of normal and of dwarf mice both in the non-fasting state and after starvation for 48, 72, and 96 hours. It is obvious that fasting for even 4 days had no greater effect upon the blood sugar of the dwarf mice than upon that of the normal control animals.[2] Among the animals starved for 48 hours, age had no influence on the fasting values.

[2] After our studies were in progress our attention was called to the work of Benedict and Lee (9), who found that 2 dwarf mice 7 months old withstood starvation for 7½ and 10½ days, respectively. This finding as far as it goes precludes sensitiveness to fasting of the degree seen in hypophysectomized animals. Benedict and Lee reported no blood sugar values.

In the non-fasting state the average blood sugar values of the dwarf mice were slightly lower than those of the control animals. The mean figure for the normal young mice (2 to 3 months old) was 139 mgm. in comparison with that of 127 mgm. for the dwarf mice. The difference of 12 is approximately twice the standard error, 6.6, and therefore barely significant. In the older groups (7 to 10 and 5 to 8 months of age), the value for the control animals was 135 mgm. while that for the dwarf mice was 113 mgm., a significant difference of 22 ± 4.65.

As shown in table 1, the average non-fasting blood sugar value for the younger dwarf mice (2 to 3 months old) was higher than for the older animals (5 to 8 months old). The figures were 127 mgm. and 113 mgm.

TABLE 1

Effect of fasting upon the blood sugar of normal and dwarf mice

	DURATION OF STARVATION	AGE OF MICE	NUMBER OF MICE	NUMBER OF DETERMINATIONS	BLOOD SUGAR	
					Average value	Standard error
	hours	*months*			*mgm. per 100 cc.*	
A. Normal mice	Non-fasting	2-3	16	35	139	4.4
	Non-fasting	7-10	26	114	135	2.8
	48	2-3	15	18	71	2.6
	48	7-10	27	30	74	2.8
	72	6-10	5	5	81	***
	96	6-12	6	6	82	***
B. Dwarf mice	Non-fasting	2-3	17	39	127	4.9
	Non-fasting	5-8	13	29	113	3.7
	48	2-3	14	16	67	2.9
	48	5-8	12	14	71	3.7
	72	6-10	5	5	82	***
	96	6-12	15	15	84	5.0

respectively; the difference of 14 ± 6.15 may possibly be significant. A tendency for younger mice to have higher non-fasting values was noted also in certain studies among the control group of normal animals but when all figures were averaged, no significant difference could be demonstrated. The question of the influence of age upon the blood sugar must therefore be decided by further work.

DISCUSSION. Since after a fast of 96 hours the blood sugar level of dwarf mice is no lower than that of the normal animal, the dwarf mouse cannot be considered deficient in that hormone of the anterior lobe of the pituitary gland which helps to maintain a normal glycemic level unless in the absence of this hormone some compensating mechanism exists for elevating the blood sugar and antagonizing the action of insulin. This

assumption seems unwarranted on the basis of existing evidence. The insular tissue in the pancreases of our dwarf mice seems normal in amount and histological appearance. Furthermore, if the compensatory elevation of the blood sugar level is to be attributed to activity of the adrenals, it would be difficult to explain the markedly hypoplastic condition of these glands observed both in the young dwarf mice examined by Kemp and Marx (5) and in those of the present experiment which were more than one year old (6). Therefore, the fact that the dwarf mice have no acidophil cells suggests that the contra-insular or blood-sugar-maintaining hormone of the anterior lobe of the hypophysis is secreted by either the basophil or chromophobe cells. Whether this is their function in the normal animal or whether in the absence of the acidophil cells they assume this function, it is not possible to state on the basis of our studies.

SUMMARY

Fasting for 48, 72, and 96 hours caused no greater decrease in the blood sugar of hereditary dwarf mice (which lack the acidophil cells of the anterior lobe of the pituitary gland) than in that of normal mice. Assuming that the hypophysis secretes a hormone which contributes to the maintenance of a normal glycemic level, the results demonstrate that the presence of the acidophil cells is not essential for this function.

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THE MECHANISM OF FAILURE IN THE COMPLETELY ISOLATED MAMMALIAN HEART

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The problem. The nature of the changes in the energetics of heart muscle during spontaneous failure is a problem of both theoretical and practical importance. The first question that asserts itself is whether the failing heart muscle does less work because the total energy consumption at a given initial fiber length diminishes or because the efficiency of utilization decreases. Observations of Decherd and Visscher (1) on the tortoise ventricle, studies by several workers on the mammalian heart-lung preparation (2),[1] and studies on the completely isolated mammalian heart by Daly and Thorpe (3), have been in agreement in showing that the total energy output of heart muscle is not less when it "fails," but that the proportion of the total energy which is put to useful work, that is the mechanical efficiency, declines. In opposition to these observations are the conclusions of Katz and Mendlowitz (4), who state on the basis of experiments upon the completely isolated mammalian heart that there is no loss in efficiency during failure.

The experiments reported in this paper were undertaken in an attempt to elucidate the possible reasons for the discrepancies between the results of the last named workers and those of other students of this problem. In the course of the study the probable reason for this discrepancy has been found, a new method for measuring the total oxygen consumption of the completely isolated dog heart has been developed, and direct simultaneous measurements of heart oxygen use by two independent methods have been made.

Since the first objective of this work was to account for the differences between the results of the studies of Katz and Mendlowitz (4) and those of other workers, it is necessary to examine the methods and conditions of the experiments of the several workers. Both Daly and Thorpe (3) and Katz and Mendlowitz (4) employed an arterio-venous oxygen difference method of measuring oxygen usage. Both measured the total coronary blood flow, but the latter workers sampled only the coronary sinus blood for venous oxygen determination, while the former sampled the mixed

[1] The extensive literature on this subject prior to 1936 is cited in this paper.

sinus and Thebesian vein blood. Katz and Mendlowitz apparently assumed, at least they mention no direct test of the point, that the coronary sinus and Thebesian vein bloods would have identical oxygen contents. This assumption has been subjected to critical test in the experiments reported in this paper.

The method of Daly and Thorpe (3) involves no assumptions as to identity of arterio-venous oxygen differences in the two fractions of cardiac venous blood and might therefore be expected to give the more reliable results.

The use of the heart-lung preparation for studies of cardiac energetics involves the assumption that the lungs have a substantially constant oxygen consumption, which is independent of such factors as the rate of blood flow through them. This has been found to be true (5). Measuring oxygen consumption by the closed circuit spirometer method (2) one determines the total oxygen use, so long as the oxygen saturation of the arterial blood remains constant, which has been checked by blood oxygen content measurements. Since the closed system spirometer method of cardiac metabolism measurement in the heart-lung has yielded different results from those reported by Katz and Mendlowitz with the A-V O_2 difference method in the isolated heart, it has seemed necessary to apply the former method to the isolated heart and to compare it with the A-V O_2 difference method. In this way the general validity of the closed circuit method can be checked.

All workers, including ourselves, who have employed the completely isolated mammalian heart have found that the coronary flow falls off over time. This decrease in coronary flow is ordinarily correlated with a decline in total oxygen consumption. Daly and Thorpe (3) ascribed the decrease in flow to embolism and found it could be retarded by adequate filters. Certain facts concerning the relation of blood flow to apparent and real oxygen consumption and the mechanical efficiency will be brought out in the description of results in this paper.

Experimental method. Experiments have been designed and performed to elucidate: *a*. The ΔO_2 (arterio-venous oxygen difference) for coronary sinus and Thebesian vein blood sampled simultaneously. *b*. The total oxygen consumption of the isolated dog heart collecting both coronary sinus and Thebesian blood separately and together, measuring the ΔO_2 and the volume flow. *c*. The oxygen consumption of the completely isolated heart with a Bayliss-Fee oxygenator in a completely closed circuit spirometer system. *d*. The comparison of a simultaneous measurement of oxygen consumption by the two methods, which are based on entirely different principles and agreement between which should constitute strong reason for believing both to be reliable.

Dogs anesthetized with amytal or nembutal were used in all of these

studies. Fresh, defibrinated dog's blood carefully filtered through four funnels each with triple layers of fine cotton mesh was used as the perfusion fluid in all experiments. Heart-lung preparations were first made as described by Peters and Visscher (2). Then the heart was completely isolated from the lungs as described by Daly and Thorpe (3), using a Bayliss-Fee (6) oxygenator in a completely closed circuit to aerate the blood. The general arrangement of the isolated heart with the oxygenator

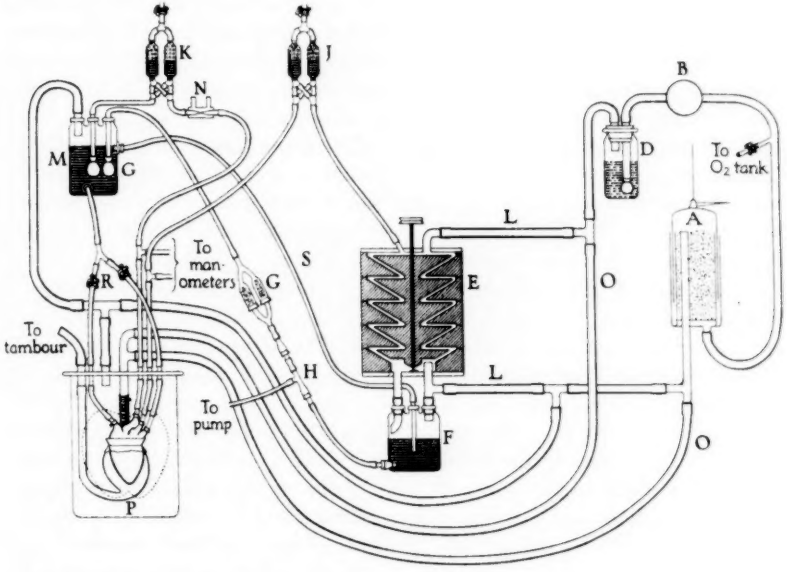

Fig. 1. Diagram of apparatus.

A, spirometer; *B*, rotary pump; *D*, Ringer's fluid to saturate air; *E*, oxygenator; *F*, collecting reservoir; *G*, filters; *H*, valves; *J*, right heart stromuhr; *K*, left heart stromuhr; *L*, position of clamps in heart-lung stage; *M*, venous reservoir; *N*, artificial resistance; *O*, position of clamps in oxygenator stage; *P*, Henderson cardiometer; *R*, screw clamps on venous return; *S*, overflow tube from venous reservoir.

and the devices for measurement of oxygen consumption, pulmonary and aortic blood pressure, and right and left heart outputs, is shown in figure 1. In the course of each circulation of the blood in this system it is made to pass through a fine cambric filter of approximately ten layers.

In experiments of the first two series after the heart was completely isolated a Morawitz (7) cannula was inserted through the right auricle and fixed in the cornonary sinus. The coronary sinus venous blood was collected under fixed pressure conditions, measured and returned to the system

through a reservoir above the oxygenator (not shown in the figure). In these cases only the Thebesian venous blood entered the right ventricle which pumped blood against a resistance through a stromuhr, back to the oxygenator. After passing through the oxygenator all of the blood was pumped by an all rubber pump back into the general reservoir from which it could return to the heart.

Samples of coronary sinus and Thebesian venous blood were collected at intervals in oiled syringes without contact with air by inserting needles through the walls of the rubber tubing carrying the bloods in question. Arterialized blood was drawn in the same way from the tube leading from the general reservoir to the heart. The oxygen contents were immediately determined in duplicate by the Van Slyke (8) manometric method. Each point in time involved three samples of blood, making six analyses. Two trained analysts could not complete more than this number per hour. Therefore we were limited to three points in any experiment, since we were unwilling to accept the error involved in having samples stand waiting for analysis for long periods. These measurements were made under the supervision of Dr. Ancel Keys to whom we express our best thanks.

In the third and fourth series of experiments the heart was completely isolated, as above, but the coronary sinus cannula was not inserted. Instead the heart was placed in the glass chamber, sometimes with and sometimes without a Henderson cardiometer, completely sealed, and the whole immersed in a large water thermostat holding the temperature at 37.5°C., constant to ± 0.005°C. The diagram in figure 1 represents the exact conditions of the experiments in this series. It is essential to hold the temperature constant in order to measure oxygen consumption by the closed-system spirometer method. It is also necessary to have the interconnecting air tubes between the general blood reservoirs, the heart chamber, and the spirometer chamber. Soda lime in the air line from the oxygenator to the spirometer bell removes carbon dioxide. Oxygen from the spirometer is moved by a rotary pump through the oxygenator and back to the spirometer. A by-pass controlled with a screw clamp determines how much oxygen is moved through the spirometer oxygenator circuit. Control experiments without the heart, with a dead heart, and without blood flow prove that the system is without leaks. Oxygen content determinations show the blood to be 99.5 per cent saturated with oxygen at the tension in the system after passing through the oxygenator, thus validating the oxygenation method. By this means one is able to measure the oxygen consumption of the entire heart, without any assumptions whatever beyond the one that the heart muscle is not building up an oxygen debt. The relative loads carried by right and left ventricles can be varied at will, within the limitation that the total pulmonary flow must be kept below 500 cc. per minute to avoid frothing in the oxygenator, which would not

invalidate oxygen consumption figures, but would cause difficulties in the return pumping of blood to the reservoir of the system as we have constructed it. An oxygenator with greater clearance than was recommended by Bayliss and Fee (6) might avoid this limitation. We have found it necessary to cover the surfaces of the oxygenator with clear lacquer in order to avoid deleterious effects of aluminum hydroxide. Fatal coronary embolism occurs unless this precaution is observed. (See Beland, Moe and Visscher (9).) The left ventricle was made to pump against a pressure of about 120 mm. Hg and the right against 25 to 45 mm. Hg.

RESULTS. A. Measurements of the oxygen content of simultaneously drawn samples of arterial, coronary sinus, and Thebesian vein blood are presented in table 1. It will be apparent that the oxygen contents of the latter two bloods are not equal, nor even approximately so except in one case out of six. The ratios between the two ΔO_2 figures, as shown in the

TABLE 1

Simultaneous coronary blood oxygen contents and arterio-venous differences

EXPERIMENT NUMBER	OXYGEN CONTENT			ΔO_2 A-V DIFFERENCE		$\frac{I}{II}$
	Arterial	Thebesian	Coronary sinus	Thebesian I	Coronary sinus II	
	cc./100 cc. blood	*cc./100 cc. blood*	*cc./100 cc. blood*	*cc./100 cc. blood*	*cc./100 cc. blood*	
Ia	11.66	9.18	7.60	2.48	4.06	0.61
Ib	12.25	8.72	7.92	3.53	4.33	0.81
Ic	12.79	10.70	10.31	2.09	2.48	0.84
IIa	19.54	15.47	15.54	4.07	4.00	1.02
IIb	19.54	15.43	16.87	4.11	2.67	1.54
IIc	19.54	14.86	16.80	4.68	2.74	1.71

last column on the right, vary between 0.61 and 1.71. Thus the assumption implicit in the Katz and Mendlowitz method, that coronary sinus blood may be taken as a *representative sample* of the whole coronary venous blood is seen to be unfounded. Calculations based on that assumption may obviously be in error by as much as 50 per cent of the total oxygen consumption. This fact invalidates any method of measuring cardiac metabolism which employs such an assumption. This would presumably be true in the intact animal and in the heart-lung, as well as in the completely isolated heart. In this regard, attention is particularly drawn to the fact that Rühl (10), who has claimed that cardiac efficiency may increase in failure, used such a method with the heart-lung. Further inconsistencies in Rühl's results are discussed elsewhere (11).

The reasons for the variations in the A-V ΔO_2 for coronary sinus and Thebesian blood have been discussed by Moe, Wood, Visscher and Keys

(12). In general it can be said that the changes in the relative proportions of blood passing through the two channels are correlated with the ΔO_2 figures.

B. The oxygen consumption of the heart was measured by collection and analysis of coronary sinus and Thebesian venous blood. Since the ΔO_2 values for coronary sinus and Thebesian bloods are different, one must obviously study both in order to measure oxygen consumption. In figure 2 we present the data from an experiment in which the two were collected and measured separately. In this experiment the ventricular

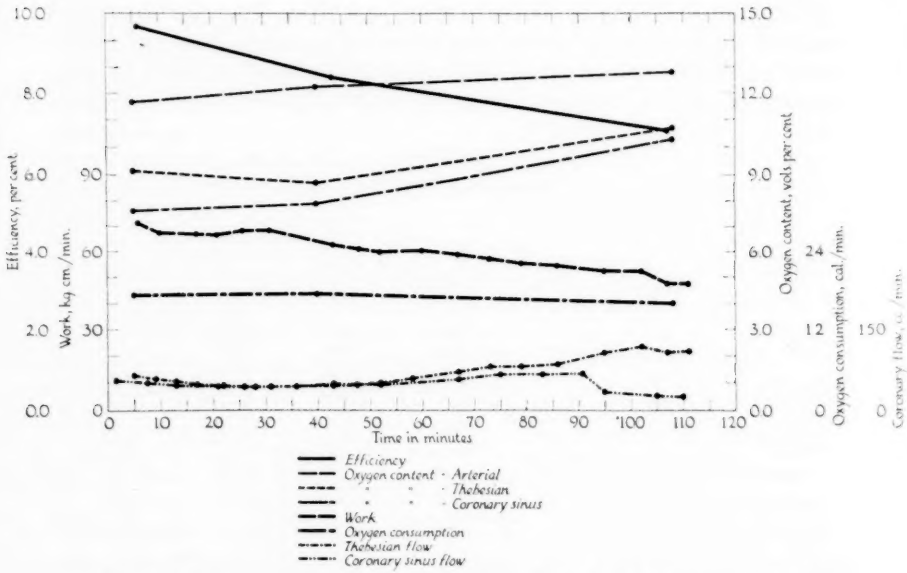

Fig. 2. Spontaneous failure in the isolated heart. Oxygen consumption calculated from coronary sinus and Thebesian vein blood measurements.

volume was not recorded, and the diastolic volume probably increased over time. Two important facts are evident. Over the course of this experiment the total oxygen consumption was very nearly constant, changing only 8 per cent, while the total work performed fell 32 per cent. The net efficiency decreased from an initial 9.7 per cent to a final 7.1 per cent. This is a decrease of 27 per cent. Figures from which the oxygen consumption curve was drawn are shown in table 2. It can be seen that, while the oxygen consumption from the Thebesian blood increased 75 per cent in less than two hours, that from the coronary sinus fraction fell 7 per cent in the same amount of time. The total change in oxygen con-

sumption is therefore small. If, however, we assume the arterio-venous difference to be the same for both fractions of the coronary blood and calculate oxygen consumption from the coronary sinus ΔO_2 and the total coronary flow, as did Katz and Mendlowitz, we get an apparent fall in O_2 consumption of nearly 20 per cent, which all but masks the actual decline in efficiency which occurred.

In these studies we have not taken into account the oxygen utilization from that fraction of the cardiac venous blood which drains into the left heart directly and is ejected through the aorta after mixing with the arterialized blood entering the left atrium. Estimates as to the proportion of the total cardiac venous blood which drains into the left heart when the latter is working against normal pressures indicate that this fraction is about 7 per cent of the total. The method of arriving at this figure will be presented in another communication. Since a portion of this left

TABLE 2

Oxygen consumption calculated from arterio-venous differences and coronary flow

TIME	I THEBESIAN FRACTION			II CORONARY SINUS FRACTION			I + II TOTAL O_2 CONSUMPTION (MEASURED VALUES)	O_2 CONSUMPTION CALCULATED ON BASIS OF CORONARY SINUS ΔO_2 ALONE (KATZ METHOD)	
	ΔO_2	Flow	O_2 consumption	ΔO_2	Flow	O_2 consumption		Total flow	O_2 consumption
min.	*vol. per cent*	*cc./min.*	*cc./min.*	*vol. per cent*	*cc./min.*	*cc./min.*	*cc./min.*	*cc. min.*	*cc. min.*
0	2.48	57.6	1.425	4.06	50	2.03	3.455	107.6	4.37
35	3.53	43.0	1.57	4.33	44	1.91	3.48	87.0	3.77
103	2.09	119.0	2.49	2.48	27	0.68	3.17	146.0	3.62

ventricular venous blood eventually passes through the coronary vessels to the right heart, the proportion of oxygen consumption of the whole heart which is missed when a method such as that employed in this paper is used is about 5 per cent. As will be noted in connection with the results given in section D of this paper, this figure is not far from that which was observed to be the difference between the total oxygen consumption measured by the spirometer method and the so-called internal method, ignoring the venous blood which enters the left ventricle and leaves via the aorta.

C. Since a continuous measurement of oxygen consumption is superior in many respects to the discontinuous observations which are possible when one employs the arterio-venous oxygen difference method just described, we have converted the oxygenator circuit into a closed system device for measuring metabolism continuously. Six complete experiments have been performed using this method. In order to illustrate the type of record obtainable we reproduce in figure 3 a segment of the original record

of one experiment. Besides the quantities graphically recorded, the heart rate and the pulmonary and aortic outputs were measured at frequent intervals, the latter by the use of stromuhrs as indicated in the description of the method. In this experiment the ventricular volume was not measured. The lowest curve D is a record of the spirometer level. It is obvious that although other quantities remained constant, the rate of fall of the spirometer was increasing over time. This increase represents an augmentation in the rate of oxygen consumption. In this experiment the heart failed very rapidly. The measured work and oxygen consumption and the computed efficiencies are plotted in figure 4 for a similar experiment in which the diastolic ventricular volume was held constant. Beginning with an efficiency of 6.7 per cent there was a fall to 3.7 per cent in 22 minutes. Ordinarily the decline has not been so rapid. The oxygen consumption remained nearly constant over the period. Measurements

Fig. 3. A segment of the original record of the experiment graphed in figure 4. *A*, time at 30-second intervals; *B*, aortic pressure; *C*, pulmonary pressure; *D*, oxygen consumption. The manometer tracings do not have a common base line.

were not continued beyond the time indicated, because the coronary flow began to fall off.

It was recognized, however, that it would be of importance to record all of the variables in question *while the coronary flow was diminishing* in the terminal stages of an experiment. In figure 5 the results of such observations are plotted. The heart had been working in the completely isolated state for an hour previous to the time of these records. Between the times marked 24 minutes and 34 minutes, the coronary flow dropped from 150 cc. per minute to zero. The measured oxygen consumption did not fall to zero until several minutes later. This lag is inherent in the system because a volume of air equal to its total air volume must move through the oxygenator several times in order that all the CO_2 shall be removed. After the 24 minute point the oxygen consumption, and particularly the calculated efficiency are purely fictitious. Till that point there is no reason

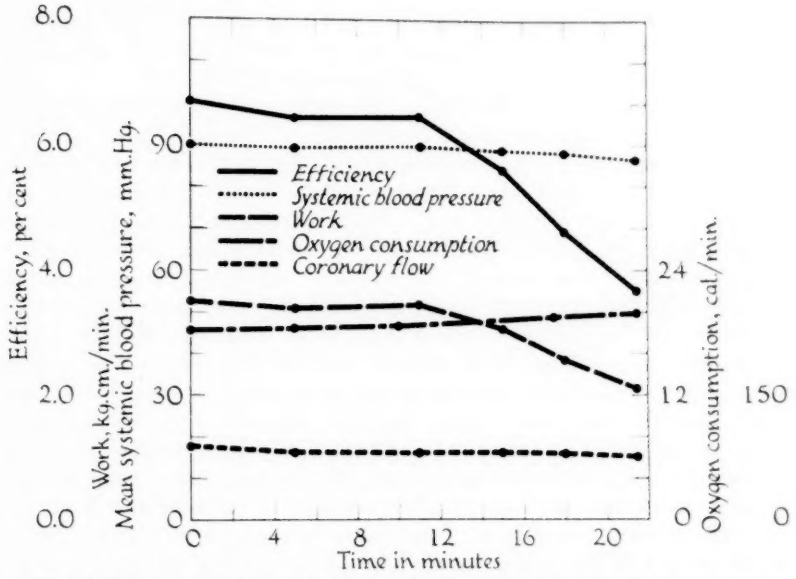

Fig. 4. Spontaneous failure in the isolated heart at constant diastolic volume. Oxygen consumption measured by the closed circuit method.

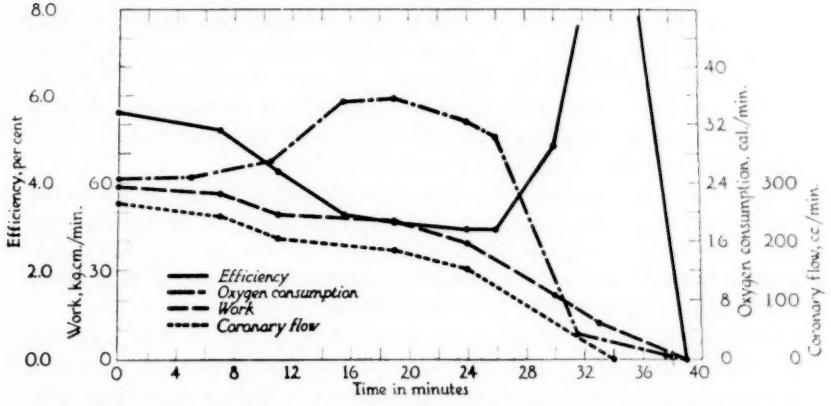

Fig. 5. Analysis of terminal stages of failure with coronary occlusion, oxygen consumption measured by the closed circuit method.

to doubt the accuracy of the figures obtained. The decrease in efficiency prior to that time is undoubtedly real, but the rising efficiency thereafter represents simply anaerobic contraction and is meaningless. Efficiencies

calculated from oxygen consumption depend for their validity upon the assumption that aerobic processes are at equilibrium with anaerobic processes, which is not correct under these circumstances.

D. The final critical question in this study was whether the oxygen consumption as measured by the arterio-venous oxygen difference method would be found to agree with that determined by the closed system spirometer method. In figure 6 and table 3 are presented the results of an experiment in which this was tested. In this experiment the total coronary venous blood was collected after ejection from the right ventricle, into which no other blood entered. In addition, continuous measurement of oxygen use was made by the spirometer method. Corrections were made, of course, for the volume of blood removed for analysis. Measurements upon the blood collected from the right ventricle showed that the oxygen consumption was steadily increasing slightly as the heart dilated in failure (the diastolic ventricular volume was not kept constant), while simultaneous continuous measurement of oxygen use by the spirometer method showed the same direction of change. The former values were on the average lower than the latter by 3.5 per cent. This difference is in the direction predicted previously in this paper on the basis of the fact that a small fraction of the cardiac venous blood enters the left ventricle directly. The magnitude of the difference found agrees with that predicted. A better agreement is not to be expected when there is an over all error of ± 2 per cent in each method.

The substantial quantitative agreement between the results of these two independent methods of measuring the cardiac oxygen consumption appears to be particularly significant. It adds considerable weight to previous evidence indicating that the closed circuit spirometer method of metabolism study is valid and that the conclusions drawn by many workers from results of such studies are essentially sound.

It is of interest to note again that the experiment in figure 6 shows the usual decrease in efficiency over time; over the course of 40 minutes, the mechanical efficiency declined from 10.2 per cent to 7.9, which is approximately the rate of decline in efficiency which has been observed in many experiments with the heart-lung preparation.

Discussion. These studies have shown in general that it is improper to use collections of coronary sinus blood as random representative samples of the whole cardiac venous blood, because the coronary sinus blood may have either lower or higher coefficients of oxygen utilization than the Thebesian blood. This fact invalidates any study using coronary sinus collection in which the mechanical conditions of the heart change during the course of time as they do under any circumstances in which such a method would be likely to be used. This defect is believed to account for the disagreement in the results of those workers, for example Rühl (10)

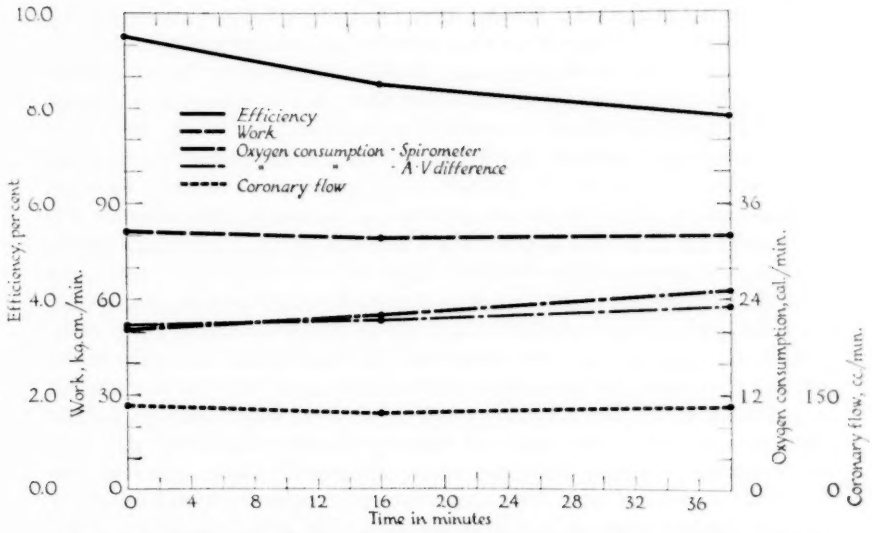

Fig. 6. Spontaneous failure in the isolated heart. Oxygen consumption measured simultaneously by the "internal" and "external" methods.

TABLE 3

Simultaneous determination of oxygen consumption by "internal" and "external" methods, total work, and efficiency (calculated from the means of the 2 values for O_2 consumption)

TIME	OXYGEN CONSUMPTION		MEAN BLOOD PRESSURE		TOTAL OUTPUT		WORK		EFFICIENCY
	A-V diff.	Spirometer	Aortic	Pulmonary	Left ventricle	Right ventricle	Left ventricle	Right ventricle	
min.	*cal./min.*	*cal./min.*	*mm. Hg*	*mm. Hg*	*cc./min.*	*cc./min.*	*kgm. cm./min.*	*kgm. cm./min.*	*per cent*
0	20.6	20.2	113	35	475	133	75.2	6.3	9.5
16	21.3	22.05	113	40	458	121	72.3	6.9	8.5
38	23.0	25.0	112	45	456	132	71.6	8.4	7.8

TIME	OXYGEN CONTENTS				ΔO_2, A-V DIFFERENCE	CORONARY FLOW	OXYGEN CONSUMPTION
	Arterial		Venous				
min.	*volumes per cent*		*volumes per cent*		*volumes per cent*	*cc./min.*	*cc./min.*
0	20.95 21.08	21.02	18.15 17.67	17.91	3.11	133	4.13
16	20.82 20.88	20.85	17.33		3.52	121	4.25
18	21.2 21.03	21.12	17.60 17.65	17.63	3.49	132	4.60

and Katz and Mendlowitz (4) who were not aware of the fact that coronary sinus and Thebesian bloods were not identical as to oxygen content.

When one measures the total oxygen consumption of the isolated heart by the spirometer method, one finds that the essential change in energetics during spontaneous failure is as was found previously in the case of the isolated tortoise ventricle and in the case of the mammalian heart-lung preparation, a decrease in mechanical efficiency. The same essential change is noted when the so-called internal method of oxygen consumption is employed and account taken of the Thebesian and coronary sinus blood.

One point in connection with the completely isolated heart-oxygenator circuit should be mentioned. In this circuit, it is difficult if not impossible to avoid a progressive acapnia as a result of which the blood pH rises to highly abnormal values. When one uses the lungs for aeration of the blood on the other hand, the total ventilation may be so adjusted as to maintain a physiological carbon dioxide tension. The effects of acapnia on the dynamics of heart muscle have not been adequately studied and therefore the importance of this defect cannot be estimated. However, after considerable experience with both the heart-lung preparation and the completely isolated heart, it is our opinion that the former is easier to use and that, except when it is necessary to isolate the right and left heart circuits entirely, as much information can be obtained with the heart-lung preparation as with the completely isolated heart, especially if a stromuhr is put in the pulmonary circuit (13).

SUMMARY AND CONCLUSIONS

1. The coefficients of oxygen utilization for coronary sinus and Thebesian vein bloods are widely different and independently variable.

2. The implicit assumption that coronary sinus blood is a representative sample of the total coronary venous blood, made in calculations of cardiac oxygen consumption by workers collecting coronary sinus blood and measuring or calculating total coronary flow is unsound, and therefore such calculations must be in error.

3. It is shown that if one collects both coronary sinus and Thebesian vein blood, and measures the arterio-venous oxygen differences, and from them determines the oxygen utilization over time, the isolated heart which is failing spontaneously shows a progressively falling efficiency of doing work.

4. Measuring oxygen consumption in the completely isolated heart by a closed circuit spirometer method, it is found also that the mechanical efficiency falls progressively in failure.

5. Simultaneous measurements of cardiac oxygen consumption by two independent methods, the total coronary arterio-venous oxygen difference method and the closed circuit spirometer system show satisfactory agree-

ment. The former method gives values which average 3.5 per cent less than the latter, which is in agreement with the fact that a small proportion of the total coronary venous blood passes via Thebesian veins into the left ventricle and was ignored in these studies.

6. The validity of previous observations on the heart-lung preparation using the closed circuit spirometer method of oxygen consumption measurement has been confirmed by showing that quantitatively similar results are obtained when one studies the completely isolated heart under proper conditions.

7. The conclusion of Katz and Mendlowitz that the isolated mammalian heart fails without loss in efficiency has been shown to be erroneous, their error arising because they ignored the fact not previously known but now demonstrated that the coronary sinus and the Thebesian vein bloods are not equal in oxygen content or in their proportionate contribution to the oxygen supply of the heart.

8. New experimental evidence has been presented showing that the foremost change in the energetics of the mammalian heart in spontaneous failure is a lowered mechanical efficiency.

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THE INFLUENCE OF POSTURE ON SKIN AND SUBCUTANEOUS TEMPERATURES

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The most fundamental alteration which occurs in changing from the recumbent to the standing posture is the sudden shifting of a column of blood, the length of the body, from a horizontal to a vertical position where it exerts a hydrostatic pressure. Blood tends to accumulate in the parts of the body below the level of the heart (1), the stroke and minute volumes of the heart decrease (2) and various compensatory mechanisms are brought into play in an effort to maintain an equitable distribution of the circulating blood. The importance of vasoconstriction in the splanchnic area in the readjustment to the upright position has been clearly demonstrated by Hill and Barnard (3). In the present work we wish to present evidence of similar compensatory vasoconstriction occurring in the peripheral circulation, particularly of the extremities, as indicated by measurements of skin and subcutaneous temperatures of various parts of the body.

Skin and subcutaneous temperature measurements at a depth of from 2 to 4 mm. were made on nine young male adult subjects with normal cardiovascular systems using the loop and needle thermocouples described by Foster (4). The thermocouples were arranged in a potentiometric system so as to permit of 4 to 8 readings in 3 to 5 minutes with an accuracy of 0.2°C. Measurements were made on the dorso-medial surface of the large toe over the first phalanx, the anterior surface of the thigh midway between the knee and the hip, the lateral border of the dorsum of the hand, a point approximately 8 cm. lateral to the navel, a point about 10 cm. directly above the nipple, the side of the neck and in the temporal region of the forehead. Skin temperatures only were measured in 5 experiments, skin and subcutaneous at the same points in 10 and subcutaneous temperatures only in 18 experiments. The temperature of the room was measured by thermometers suspended at various points in the vicinity of the tilting table and was kept relatively constant (within 1°C.) by means of fans and heaters. All of the experiments were done at

average room temperatures of 25°C. or over. Wet and dry bulb temperature readings were made at intervals.

The following procedure was followed in all of the experiments. At least one hour after the last meal, the nude subject reclined on a tilting table with his buttocks resting in a shallow-grooved support bolted across the table and was kept from slipping by two padded wooden blocks secured in place over the iliac crests by a cross-bar. The subject was thus supported with a minimum impairment of the circulation and without the use of a foot-board, the absence of which eliminated muscular movements due to the unavoidable shifting of weight which occurs particularly in subjects who have difficulty in maintaining the upright position. After the various adjustments had been made, the thermocouples were fixed in their respective positions and a sphygmomanometer cuff wrapped around the arm not being used for temperature measurements. Skin and subcutaneous temperatures, blood pressure and pulse rate determinations were made at intervals until consecutive readings showed that constant resting levels had been reached. The subject was then tilted to an upright position (75°) and remained there for 14 to 40 minutes depending on his ability to tolerate this position without signs of syncope. Observations were continued for 20 to 90 minutes after return to the horizontal position. Five of our subjects showed no signs of discomfort when tilted for periods as long as 40 minutes; four consistently showed signs of syncope.

At the beginning of the experiment with the subject recumbent the subcutaneous as well as the skin temperatures showed distinct gradients from toe to forehead. At room temperatures of 25 to 27°C. these gradients usually persisted with slight variation in level as adjustment to the environmental temperature was taking place. At higher room temperatures, however, the gradients frequently tended to disappear and the temperatures of the hands and toes approached or were even higher than those of the trunk and face. Throughout the experiment there was usually a gradient from skin to subcutaneous tissue, the temperature of the latter at all points studied being 0.2 to 0.7°C. higher. In general, changes in subcutaneous temperature paralleled those in skin temperature and, while of smaller magnitude, were more consistent and less influenced by sweating and minor fluctuations in room temperature. The results of several experiments, in which skin, subcutaneous and intramuscular temperatures were recorded at the same points suggest that changes in intramuscular temperature are smaller but in the same direction as those in skin and subcutaneous temperatures.

Irrespective of the levels in the horizontal position, when the subject was tilted to 75° there occurred a sharp or gradual drop in skin and subcutaneous temperatures. In some cases, the temperatures began to fall

immediately after the upright position was assumed and usually reached their lowest values in 10 to 15 minutes, there being no further or only slight changes even though the position was maintained; in other experiments the temperatures continued to drop throughout the upright position. When the room temperature was below 27°C. the decreases in skin and subcutaneous temperatures were greatest in the toe and progressively less in the hand, thigh, abdomen, chest and forehead. At higher room temperatures, however, when, as indicated above, the initial levels of the extremities and other parts of the body were high and indicated complete

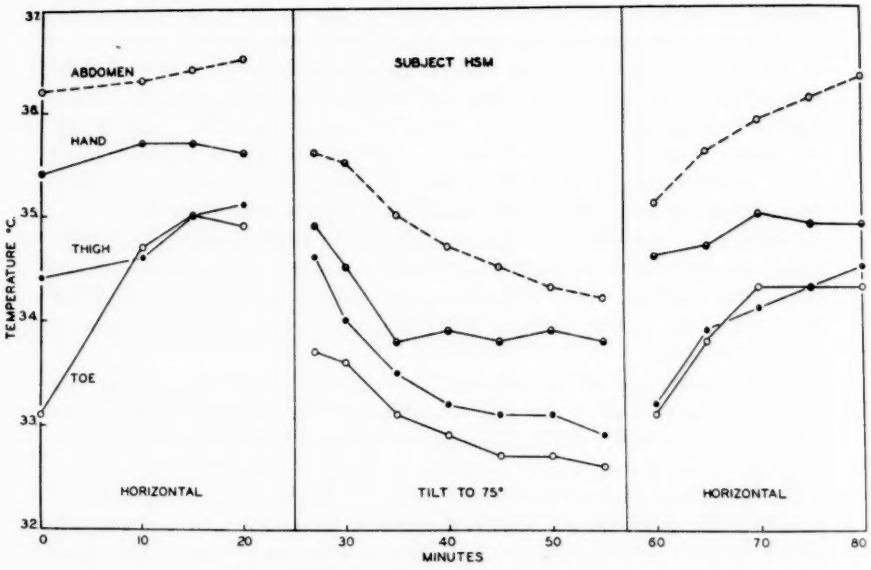

Fig. 1. Subcutaneous temperature changes in a "non-fainting" subject. Average room temperature—30.9°C.

vascular dilatation, the changes in the toe, thigh, abdomen and hand were uniform and of the same magnitude (fig. 1). On return of the subject to the horizontal position the temperatures began to rise and had returned to or were approaching their original levels in from 15 to 60 minutes, the extremities usually showing the greatest lag in recovery.

Youmans, Akeroyd and Frank (5) concluded that heating of the forearms and hands was able to overcome the drop in skin temperature of the foot which occurs in the quiet standing position. To test this point 4 experiments were performed on 2 subjects in which the left foot was heated continuously by means of an electric pad and subcutaneous temper-

ature measurements made on the right side of the body. Under these conditions complete vascular dilatation was obtained in the right foot and practically all the vasoconstrictor reflexes eliminated. Tilting, as shown in figure 2, resulted in the usual decrease in subcutaneous temperature of the right toe as well as other parts of the body. The discrepancy between our findings and those of Youmans, Akeroyd and Frank may be due to the more complete dilatation achieved in our experiments. When vascular vasodilatation is incomplete there is an interplay of the two opposing influences, that of room temperature tending to increase the tissue temperatures and posture tending to produce changes in the opposite direction. We have found that the adaptation of the skin to a given environmental temperature takes place very slowly, in some cases requiring

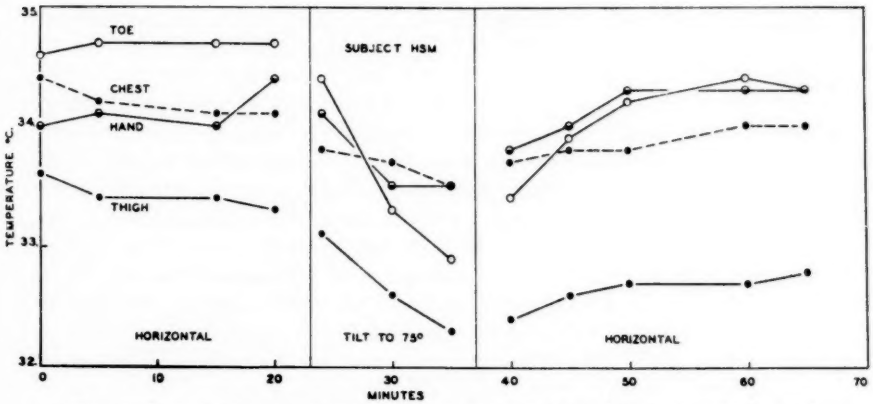

Fig. 2. Subcutaneous temperature changes in a "non-fainting" subject with reflex vasodilatation. Average room temperature—25°C.

periods of 45 to 90 minutes. If the subject is tilted before equilibrium is attained, the postural response interrupts that due to temperature as shown in figure 3. This interruption is, however, only momentary, the tissue temperatures continuing to rise until complete dilatation, is achieved. At this point, the curves are reversed and a second drop in tissue temperature of the extremities is observed. These results may also help to explain the divergence of our results and those of Roth, Williams and Sheard (6), who reported a rise in skin temperature of the toes and fingers in the upright position, the subject standing on a blanket. Their experiments in which a tilting table was used are not comparable to ours, since their subjects were tilted to angles of only 15 and 20°. We were unable to obtain consistent results at angles less than 60°, in which position the changes are smaller but in the same direction as those at 75°.

DISCUSSION. The results of these experiments indicate that, in adjusting to the upright position, vasoconstriction takes place not only in the splanchnic area (3) and in the lower extremities (5) but also in the lower part of the trunk and upper extremities, and to a lesser extent, in the chest and face. This vasoconstriction takes place promptly on assumption of the upright position and usually disappears when the recumbent position is resumed. Wald, Guernsey and Scott (7) have shown that about 10 seconds after changing from the recumbent to the standing

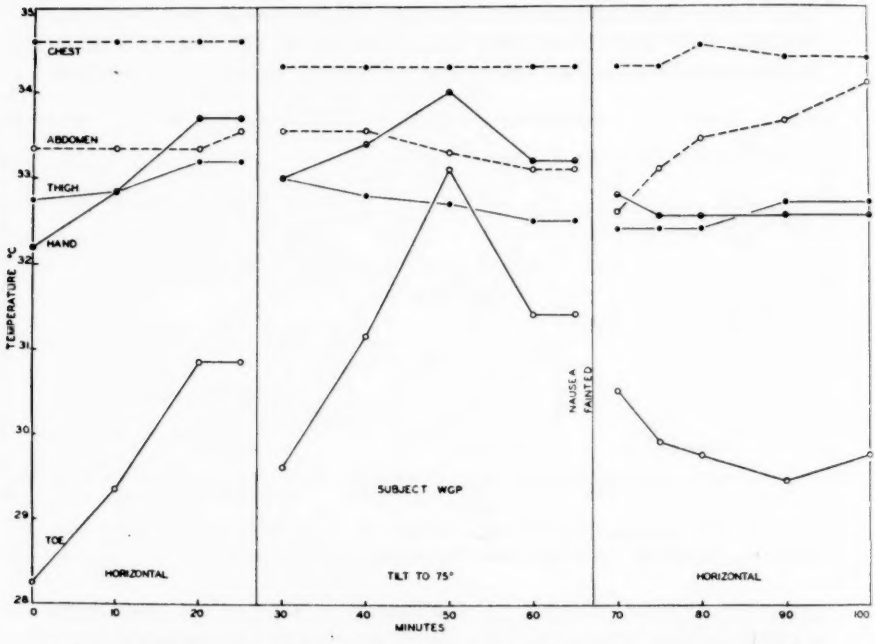

Fig. 3. Subcutaneous temperature changes in "fainting" subject tilted before temperature equilibrium had been reached. Average room temperature—26°C.

position, the systolic pressure has dropped 5 to 40 mm. Hg but returned to the recumbent level within 30 seconds. The work of these and other investigators (8) suggests that the drop in systolic pressure evokes compensatory reflexes from the sino-aortic areas leading to the generalized vasoconstriction, increased heart rate and recovery of the systolic pressure which is usually observed. Subsequent changes would be attributable to medullary ischemia. Roth (9) has recently shown that some cases of orthostatic hypotension exhibit an anhydrosis similar to that observed after extensive abdominal sympathetic denervation (ventral rhizotomy

from the sixth thoracic to second lumbar inclusive), and that in the latter condition there is a marked accentuation of the blood pressure drop in the upright position. These findings suggest, on the basis of our present experiments, that one of the fundamental disturbances in postural hypotension may be the absence of vasoconstriction, particularly in the extremities.

Our experiments have provided no explanation for the consistent inability of certain "normal" individuals to maintain the upright posture for more than short intervals of time. That this is not due to a failure of the vasoconstrictor compensatory mechanism to operate is indicated by the fact that these individuals show decreases in skin and subcutaneous temperatures (vasoconstriction) as great as, or, in some cases, greater than those of individuals who can well tolerate the upright position. The blood pressure and pulse rate changes are similar to those of non-fainting individuals until signs of syncope appear. There are evidences of simultaneous stimulation of the sympathetic and parasympathetic systems by the progressive medullary anemia as manifested by tachycardia, in addition to the vasoconstriction, occurring along with sweating, yawning and nausea. Bradycardia, evidence of vagal stimulation, develops just before syncope and in some cases is still marked for several minutes after return to the horizontal position. There is an apparent similarity between the collapse studied by us and the phenomenon described as "gravity shock" which develops if subjects who have engaged in sudden and strenuous exercise are kept immobile in the upright position. Mateef (10) concludes that the condition depends on pooling of the blood in the dilated capillary bed of the legs following the exercise. The type of collapse we observed is similar to that studied by Weiss and his associates (11) who, however, used the injection of sodium nitrite in addition to the upright position to produce it. These authors believe that the primary cause of the collapse is a lack of adequate venous tone and consequent pooling in the dependent parts. We (12) have obtained evidence of such pooling in studies on circulation time in the upright position.

SUMMARY

Assumption of the upright position is usually accompanied by a prompt and significant fall in the skin and subcutaneous temperatures of the body. At room temperatures of 25 to 27°C. the changes are most marked in the lower extremities and progressively less in the hands, abdomen, chest, neck and forehead. At high environmental temperatures the changes are uniform and of the same magnitude in the extremities and trunk. Heating of an extremity in the upright position overcomes the postural effect until complete vasodilatation is achieved at which point the postural change is again evident. No significant differences were

found in the temperature response of subjects who showed signs of syncope in the upright position and those who did not.

We are grateful to the students who acted as subjects for these experiments and to Dr. H. M. Sweeney for assistance in designing the tilting table.

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THE INFLUENCE OF POSTURE ON CIRCULATION TIME

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We have earlier presented evidence that in the upright position there is a decrease in cardiac output (1) in spite of compensatory vasoconstriction in the extremities (2). This suggested the desirability of investigating the circulation time in the lower extremities under similar experimental conditions.

Least circulation times were determined in 25 experiments on eleven normal male subjects, ranging in age from 20 to 38 years, by the method of Spier, Wright and Saylor (3). This test depends on the time necessary for a definite localized sensation of heat to be produced when the solution (containing magnesium sulphate, calcium gluconate and copper sulphate) is carried in the blood stream from the site of injection to various points in the vascular system. The nude subject rested on the tilting table previously described (2) for at least 20 minutes. Frequent blood pressure and pulse rate determinations were made during this and subsequent intervals. At the end of the rest period 2 cc. of the test solution were injected into one of the foot veins (great saphenous or one of its tributaries) or into an antecubital vein of the left arm supported at heart level. The subject was asked to report when he felt a warm sensation in the tongue, perineum, right and left hand and right and left foot respectively, the times being recorded by a stopwatch. The subject was then allowed to rest for 20 minutes, the table was slowly tilted to an angle of 75 degrees and the injection repeated 3 to 13 minutes later, depending on the ability of the subject to tolerate the upright position. Seven of our subjects showed signs of oncoming syncope after 5 or 6 minutes at which time they were returned to the horizontal position. In some cases syncope intervened so rapidly as to make it impossible to test the subject. In general, if no reactions were reported at the end of 3 minutes after the injection, the subject was returned to the horizontal position and asked to report if and when he perceived any sensations.

The data of 5 representative experiments are given in table 1. In only two of our subjects were we able to obtain circulation times in the

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upright position within 3 minutes after the solution was injected into a foot vein. In one subject (table 1, H. M. S.) the circulation times were about twice as long as the corresponding times in the horizontal position, in the second subject they were over 3 times as long. In every case, these subjects reported a second series of sensations on being returned to the

TABLE 1

Influence of posture on circulation time (method of Spier, Wright and Saylor)

SUBJECT	VEIN	POSITION	TIME INJECTED AFTER CHANGE IN POSITION	REACTION TIME (SECONDS)					
				Tongue	Perineum	Right hand	Left hand	Right foot	Left foot
			min.						
H. M. S.	Foot	Hor.		20	38	34	34	47	47
		75°	13	58	65	60	60	70	70
		Hor.		125 (5)*	134 (14)	127 (7)	127 (7)	137 (17)	137 (17)
L. A. T.	Foot	Hor.		38	47	53	53	68	68
		75°	6						
		Hor.		194 (14)	201 (21)	203 (23)	203 (23)	220 (40)	220 (40)
D. R. U.	Foot	Hor.		40	54	56	52	66	71
		75°	4.5†						
		Hor.		190 (10)	205 (25)	197 (17)	198 (18)	206 (26)	207 (27)
H. S. M.	Foot	Hor.		26	35	33	33	44	44
		75°	6	190	210	234	234	252	252
		Hor.		314 (14)	323 (23)	317 (17)	317 (17)	330 (30)	330 (30)
H. S. M.	Arm	Hor.		16	27	29	29	46	46
		75°	11	16	22	30	30	71	73
E. G. N.	Foot	Hor.		36		77			
		75°	6†						
		Hor.		89 (19)	205 (116)‡	190 (101)‡	190 (101)‡		

* Values in parentheses indicate number of seconds after return to horizontal position.

† Signs of oncoming syncope during test.

‡ Leg raised approximately 12 inches at 180 seconds after injection and 110 seconds after return to horizontal position.

horizontal. In 11 of the experiments no sensations were obtained in the upright position within periods (89 to 221 seconds) which in every case well exceeded the longest reaction time for the subject in the horizontal position (table 1, subjects L. A. T. and D. R. U.). Milking of the vein immediately after the injection did not affect the response. Since the pharmacodynamic effect of any substance depends on its effective con-

centration, the above findings suggest a dilution of the test solution in the blood, which, under the conditions of our experiment, must be due to either the accumulation of an increased volume of blood in the legs or a diminished rate of blood flow, or to both. That the failure to obtain any sensations in these cases in the upright position is due particularly to marked slowing of the rate of venous return is strikingly attested by the fact that in every case these subjects reported a series of strong subjective sensations within 10 to 41 seconds after being returned to the horizontal position. These results are, in general, similar to those of Thompson, Alper and Thompson (4) who used brilliant vital red and of Bock, Dill and Edwards (5) who used the histamine method. The latter investigators failed to obtain reactions in one case even when double the usual amount of histamine was injected with the subject in the "quiet standing" position. They also report a case in which no reaction was obtained after 5 minutes of standing, but a flush appeared 25 seconds after the subject lay down.

In two experiments the subject was kept in the upright position for longer than the usual 3 minute period after the injection. In the first of these experiments circulation times were obtained only to the tongue and perineum while the subject was upright and these were over 5 times as long as in the horizontal position. He was returned to the latter position at the end of 5 minutes and soon after reported sensations in the other parts of the body. In the second experiment (table 1, H. S. M.) the circulation times were over 6 times as long as in the horizontal position. On the return of the subject to the horizontal position 5 minutes after the injection there followed a second and more pronounced series of sensations. Bock, Dill and Edwards found the circulation times (ankle to face) to be 1.5 to 4.5 times longer in the standing than in the recumbent position. Youmans, Akeroyd and Frank (6) found a maximum difference of 4 times, using the sodium cyanide, sodium hydrocholate or saccharin methods (leg to carotid) on 3 subjects. Our maximum changes are larger, probably because of the fact that our subject was passively tilted and muscular movements of the lower extremities were absent.

The reporting of a second series of sensations on the return from the upright to the horizontal position is of interest, since it never occurred in any of the experiments previous to the tilting. These findings suggest that although the blood flow in the upright position was sufficient in these cases to carry the solution in effective concentration to the heart and out to the peripheral circulation to produce a response, a considerable amount of the solution still remained in the venous channel. The return to the horizontal position, accelerating the venous return by eliminating the influence of gravity, resulted in a washing of the remaining solution toward the heart and a second and usually stronger set of sensations. The experiment on subject E. G. N. shown in table 1 illustrates this even more

definitely. The subject reported sensations in the tongue and right hand in the horizontal position after injection of the test solution in the left foot vein. He was then tilted to 75 degrees and a second injection made 6 minutes after. Seventy seconds later he showed signs of oncoming syncope and was returned to the horizontal, having reported no sensations in the upright position. Nineteen seconds after being returned to the horizontal position he reported a sensation in the tongue but none in the right hand or other parts of the body. At the end of three minutes the left leg was raised about 12 inches from the table. Ten seconds later the subject reported distinct sensations in the right and left hands and after 25 seconds in the perineum.

That the decrease in the velocity of blood flow in the upright position is primarily due to pooling in the veins, particularly of the lower extremities, is further suggested by the results of the experiments in which the test solution was injected into an antecubital vein at heart level. In these cases (table 1, subject H. S. M.) the circulation times in the upright position were the same or only slightly increased; in several instances, the circulation times to various points were actually shorter. The differences between these findings and the results of the experiments in which the injections were made into the foot vein may be explained as being due to the influence of gravity in retarding the flow of the solution to the heart in the longer length of vein.

We have recently shown (2) that in the upright position there is a decrease in skin and subcutaneous temperature in the periphery, particularly manifest in the extremities. This suggests that one of the mechanisms for adjustment to the upright position is a vasoconstriction and slowing of the volume flow in the dependent parts, thus preventing a flooding of the capillary reservoirs. Such a compensatory mechanism is only partially effective, however, for once the blood succeeds in getting through to the veins, it has difficulty in returning to the heart as manifested by the marked increase in circulation time and by the decrease in cardiac output. In this way a vicious cycle is created. The volume flow is slowed not only as a result of the difficulty in returning blood to the heart against gravity but also by the compensating mechanism itself. Usually, this slower blood flow is within the limits of safety and provides sufficient return to satisfy the demands of gas transport. The difficulty experienced by some of our subjects in maintaining the upright position for even short periods of time may be due to a slowing beyond these limits due to a lack of venous tone (7).

SUMMARY

Measurements of the circulation times in the upright (75°) position indicate a marked retardation of blood flow in the lower extremities. This

slowing appears to be due primarily to stagnation or pooling in the veins of the extremities.

The authors express their appreciation to the individuals who acted as subjects for these experiments.

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GONADOTROPIC EXTRACTS FROM GREEN LEAVES

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Qualitative or quantitative restriction of dietary protein is known to interfere with reproduction even when the dietary regime is sufficient for maintenance and for some growth (1, 2, 3, 4). In the rat, this disturbance is manifested by an abrupt cessation of the vaginal cycle, and the suspension of ovulation and the ovarian cycle as well. Although several other types of dietary deficiency are known to produce some aberration of the oestrous cycle, only protein deficiency appears to be able to produce the syndrome noted above.[3] This syndrome bears a significant resemblance to the effects of hypophysectomy. Because of the protein nature of the pituitary hormones it seemed profitable to entertain the hypothesis that dietary proteins contain indispensable precursors for the formation of the pituitary hormones. Indeed, the marked influence of protein restriction on growth and lactation enhanced the attractiveness of such hypothesis.

A simple mixture of whole wheat, alfalfa leaf meal and soybean meal had proven to be entirely adequate for reproduction in the rabbit (5). If the above hypothesis were correct, the postulated precursors should be present in one or more of these dietary ingredients. Moreover, the possibility existed that the hypothetical precursors occurred in nature in a form closely related to the chemical constitution of the pituitary hormone, and in that event, would possess some gonad-stimulating capacity. With such possibilities in mind, extraction experiments were undertaken. Because wheat as a sole source of protein is inadequate for reproduction (1), and because green leaves have long been suspected of stimulating reproduction, it was decided to use the alfalfa leaf for the first experiments.

Success came early in this attempt (6) but the commercial alfalfa leaf meal proved very unreliable as a source. Since it was impossible to secure adequate information concerning the history of these commercial

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[2] This work was supported by a grant from the Rockefeller Foundation.

[3] Vitamin B deficiency and complete starvation also produce this syndrome, but they both involve decreased protein intake.

samples in regard to conditions of growth, maturity at the time of cutting, etc., we were unable to make further progress with this material. The work of Schnabel on the biological properties of immature oat grass (7) suggested the use of this plant for our experiments. It was found that immature oat grass regularly yielded potent extracts (8). Although it is not yet possible to define the optimal conditions for the production of the plant gonadotropins, sufficient information is now available to prescribe a source material and an extraction procedure which will enable other investigators to secure potent gonadotropic extracts without difficulty.

METHODS. The extraction procedure is based upon the Katzman-Doisy benzoic acid method for pregnancy urine (9). One kilogram of the dried leaf is suspended in about 12 liters of water and sufficient 3N NaOH is added to bring the pH to about 8.4 (phenolphthalein). For the first hour more base is added at 15 minute intervals in order to maintain this pH. At the end of 4 hours, during which time the material has been stirred frequently, the extract is filtered, either through a mat of moistened alfalfa meal or very coarse filter paper (Schleicher and Schull, 520 B) on a Buchner funnel. The retained fluid is expressed from the residue in an ordinary cider press through unbleached muslin, and is combined with the filtrate.

To this filtrate, strong sulphuric acid is added to adjust the pH to about 5.0 (brom-cresol purple and brom-cresol green, external indicators). Then, with vigorous stirring, 100 cc. of a saturated solution of benzoic acid in acetone are added for each liter of filtrate. After settling, the precipitate is separated by filtration, washed twice with saturated aqueous benzoic acid (chilled) and twice with 5 volumes of chilled acetone, and then air-dried on the filter.

The air-dried precipitate, now weighing about 100 grams, is extracted for 4 hours with 800 cc. of 30 per cent (by volume) acetone adjusted to pH 8.4, and then centrifuged. The residue is again extracted in an identical manner and the fluids from the 2 extractions combined.

The combined fluids are adjusted to pH 6.0 (brom-thymol blue) and poured into 8 volumes of chilled acetone. If precipitation does not occur readily, strong sulphuric acid (3N) is added with vigorous stirring to complete the process. The centrifuged precipitate may then be dried, powdered, and stored until used. At this stage the product from 1 kilogram of dried leaf weighs about 10 grams. Just prior to injection the powder may be extracted for 1 hour in water at pH 7.4. Considerable insoluble material may be removed by centrifuging, and the resulting dark brown solution used for biological testing.

In the early experiments the material was injected either intra-peritoneally or intravenously into sexually mature rabbits and the result determined by examination of the ovaries 48 to 72 hours later. For the last

2 years, however, all tests have been performed by the intravenous injection of the solution into post-partum rabbits, and the presence or absence of ovulation determined in 18 to 24 hours.

RESULTS. A. *Alfalfa leaf meal.* Active extracts were prepared from 11 of 28 samples of alfalfa leaf meal examined. The quantity of original material required to furnish one ovulating dose varied from 175 to 750 grams. Repeated attempts (57) were made to secure some biological activity from the 17 inactive samples, including some extractions with aqueous pyridine, and some precipitations with tannic acid, phosphotungstic acid, and Lloyd's reagent. In no case did alteration of the extraction technique alter the result. Because of our inability to obtain any information regarding the history of most of the samples, we are unable to make any correlation between biological activity and any other property. Two samples of known history were obtained from Kansas.[4] These were cut when the plants were about 4 inches high and were artificially dried. Both of these samples yielded potent extracts.

B. *Immature oat grass.* For 4 successive years we have been able to obtain consistently potent samples of oatgrass from crops grown either in Kansas or in New Jersey. The plants were cut when 4 to 6 inches tall and either sun cured in the field, or dried in a commercial drier. One Kansas cutting and 2 New Jersey cuttings were divided into 2 parts, 1 of which was sun cured, the other dried by machine. Although active extracts have been obtained from grass cured in either manner, the sun cured product has thus far yielded more gonadotropic activity per gram of dried leaf. The only sample of oatgrass which has failed to show biological activity when tested by our methods was a machine dried product from Kansas. Nevertheless our data do not permit any definite statement as to the effect of heat drying upon the biological activity.

With the extraction procedure outlined in this paper, one ovulating dose of extract may be obtained from 30 to 150 grams of dried oat leaf.

C. *Other materials.* A few extractions have been performed on samples of immature Sudan grass; timothy, barley and rye grass. No biological activity was found in any of these. Confirming Fevold, Hisaw, and Greep (10), we were successful in preparing potent extracts from some samples of yeast,[5] but not from those ordinarily available commercially. With this material the benzoic acid procedure gave results at least as good as the aqueous pyridine method recommended by Greep and co-workers.

D. *Properties of the biologically active substance.* Any statement concerning the chemical or biological properties of the active substance must be tempered by the realization that the minute amount of activity has

[4] American Butter Company, Kansas City, Missouri, through the courtesy of Dr. Charles Schnabel.

[5] Top yeast, kindly furnished by Esslinger Brewery, Philadelphia, Pa.

been examined in the presence of enormous amounts of impurities. With this qualification in mind, it is possible to state that the activity is soluble in water, 30 per cent (by volume) alcohol, or acetone, at hydrogen ion concentrations slightly above and below neutrality. It is insoluble in 90 per cent acetone or alcohol, in butyl alcohol, chloroform and ether. It is precipitated from acid solutions by benzoic acid and tannic acid. The active material deteriorates at room temperature, either in the dry form or in solution at pH 7.0. Considerable loss of activity occurs even when the dry material is stored in the refrigerator in stoppered containers.

E. *Deterioration of the activity in the dried leaf and the development of toxicity.* When the plant product is first received in the laboratory it is possible to prepare highly potent extracts which possess very little toxicity in the effective doses. With the passage of time, however, the original effective dose becomes increasingly toxic, and more and more of the extract is required to produce ovulation in the test animals. Observations on 4 samples suggest that toxicity develops as a result of exposure to possible contamination from the outside. Two samples were stored throughout one entire summer at room temperature in the closed containers in which they had been received. When these samples were opened in the following autumn, they yielded potent extracts of very low toxicity. Two other samples, cut from the same field at the same time, were stored under identical conditions, but the containers were opened from time to time to obtain small amounts of material for extraction purposes. Both of these latter samples showed the usual deterioration and the development of toxicity.

Assay of the biological activity is impossible without the removal of the toxicity. This may be accomplished in several ways, the most convenient being fractionation with chloroform at pH 6.0.[6] A solution of the final powder may be adjusted to this pH and shaken with a small amount of chloroform. Upon centrifuging, a gelatinous mass separates out in the chloroform, leaving a less toxic supernatant fluid containing the activity. Another way of removing toxicity is by re-extraction of the final powder with 30 per cent acetone at pH 7.0 and precipitation from 8 volumes of chilled acetone at pH 6.0. It is impossible to state whether any loss of activity is entailed in such procedures of purification.

Discussion. Despite the fact that the presence of gonadotropic activity in green plants is in harmony with the hypothesis which prompted this investigation, we fully realize that this finding can not be regarded as significant support for the hypothesis. From the observed chemical properties and from the pronounced instability at low temperatures, it is obvious that the plant gonadotropin bears some resemblance to the pituitary

[6] This procedure was suggested to us by Doctor Gurin, of the Department of Physiological Chemistry, University of Pennsylvania Medical School.

hormone. Yet, until we know more of the mode of action of the plant product, very little stress can be placed upon this resemblance. Still less can be said regarding the physiological importance of these plant gonadotropins. They may be merely pharmacological oddities.

On the other hand, it should be noted that some of the samples which furnished the active gonadotropic extracts also furnished the orally active growth factor[7] of Kohler, Elvehjem, and Hart (11). Whether the gonadotropic material from plants will show any physiological effect upon oral administration remains for future work to decide. The crucial test of its physiological importance can be made only when we are able to produce pituitary hypofunction by specific dietary restriction, and test the ability of the plant gonadotropin to remedy this deficiency by oral administration. Before such experiments can be conducted a great improvement must be made in the production of the plant product.

SUMMARY

It is possible to extract from the dried leaves of immature grasses a fraction which is capable of producing ovulation upon injection into rabbits. The observed chemical properties of this fraction and its pronounced instability at low temperatures simulate the properties of the gonadotropic material in anterior pituitary extracts.

During the course of this investigation continued help was received from Dr. Charles F. Schnabel of the American Butter Company who kindly supplied many of the active samples. To Dr. Walter Russell, of the New Jersey State Agricultural Experiment Station, we are particularly indebted, for his invaluable coöperation. It was through his efforts that we were able to obtain from New Jersey immature grasses of specified age and method of drying.

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AN EXAMINATION OF THE HYPNOTOXIN THEORY OF SLEEP

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The hypnotoxin theory of sleep was advanced by Pieron in 1913 (1). His evidence of most physiologic interest was the occurrence of depression and somnolence in some of his normal dogs in which from 4 to 6 cc. of cerebrospinal fluid was replaced by an equal volume of cerebrospinal fluid from fatigued dogs or of alcoholic extracts and dialysates of the blood serum or cerebrospinal fluid of fatigued dogs. Depression or somnolence was observed only when the substances from the fatigued animals were introduced into the ventricles of the brain. We believe that a critical reader would consider the control experiments inadequate. Yet, some of Pieron's observations indicated that a "hypnotoxin" was present in cerebrospinal fluid.

No reference could be found in the literature pertaining to the repetition of Pieron's observations.

We have repeated those of Pieron's experiments which we considered more physiologic and have in addition performed many control experiments in an attempt to analyse the Pieron phenomenon which undoubtedly occurs.

METHODS. Healthy vigorous dogs were kept constantly awake in a standing position for periods varying from 7 to 16 days, or two or three days after the appearance of "fatigue anorexia."

The rectal temperature of the dogs deprived of sleep was determined. The average temperature before the onset of "fatigue anorexia" was 102°F. The rectal temperature of the dogs at the time their cerebrospinal fluid was removed averaged 104.2°F., an average elevation of 2.2°F. The temperature does not become elevated until the development of "fatigue anorexia." The body weight of the dogs used in our study varied from 20 to 30 pounds. Aseptic cisternal puncture was performed on these unanesthetized sleepy and fatigued animals. Eight cubic centimeters of clear "fatigue cerebrospinal fluid" were withdrawn slowly. While this "fatigue fluid" was being withdrawn into a warmed syringe, 8 cc. of cerebrospinal fluid were being withdrawn from a healthy, vigorous animal which had been sleeping and resting normally. The "fatigue cerebro-

spinal fluid" was then injected into the cisterna of the latter animal. The removal and replacement of fluid was performed slowly over a period of 3 minutes. It should be added that, using the trained and coöperative animal and after the acquisition of skill, cisternal puncture on the trained dog is a simple procedure. Blood tinged fluid was obtained in only four out of over two hundred cisternal punctures. Experiments in which blood tinged fluid was obtained were discarded. The temperature of the fluid was maintained at all times.

Various types of control experiments were performed for varying reasons, and will be described later. We did not inject blood serum from the fatigued animals into the ventricle of normal animals, as Pieron did, because there is evidence showing that serum or blood causes meningeal irritation (2).

Observations were made in regard to behavior, depression and respiration. The rectal temperature was recorded every half hour. *The depression of the animals was graded as follows: 1 plus*, slight but definite depression, the animal being less active and lively than normal; *2 plus*, moderate depression; the dog lies down, is much less alert than normal and responds only on being urged to do so; he will not eat; *3 plus*, severe depression; the animal lies on one side, is inactive and apparently asleep, is quite unresponsive but opens eyes when called loudly, stands when stood up, but soon lies down and apparently sleeps; *4 plus*; "sleep," marked "somnolence," difficult to arouse, but can be aroused, immediately slumps to floor when stood up, like a child sleeping very soundly.

To ascertain whether the fluid we injected into the cisterna after the withdrawal of cerebrospinal fluid reached the lateral ventricles, we withdrew 3 cc. of cerebrospinal fluid in 3 dogs and replaced it with 3 cc. of cerebrospinal fluid colored with methylene blue. Two of the animals were killed at 5 minutes and one at 15 minutes. At 5 minutes the dye had stained the foramina of the lateral ventricles; at 15 minutes the dye was found in the lateral ventricles.

The "fatigued" dogs go to sleep within five minutes after the withdrawal of the cerebrospinal fluid; the sleep is profound since they are aroused with difficulty and lie down immediately after being stood up. They react like normal dogs which show a 4 plus depression after receiving "fatigue" cerebrospinal fluid.

Results. The results on 20 of the animals which received "fatigue fluid" and on the 48 animals which served as various types of controls are summarized in table 1.

Group I. *The effect of withdrawal of 8 cc. of fluid and its replacement with 8 cc. of fluid from a fatigued animal.* As a rule when a normal animal received "fatigue fluid," we had another animal which had received "normal fluid" to serve as a comparative control.

Among the first six dogs which received the "fatigue fluid," two showed a 4 plus depression and three a 3 plus depression. The phenomenon was so striking, that a moving picture (dogs 2 and 5 and control dog 6) was prepared and demonstrated (3). As our work was extended the Pieron phenomenon was found not to be a simple one.

A description of the response of the three dogs which manifested a 4 plus depression on having 8 cc. of their cerebrospinal fluid replaced by 8 cc. of "fatigue" cerebrospinal fluid follows: The dog is normal and lively for 20 or 30 minutes. After this preliminary period the nictitating membrane moves slowly over the cornea and the eyelids close to be suddenly opened with a start. At 45 minutes the animal is still more depressed and

TABLE 1

Effect of intraventricular procedures upon the normal dog

GROUP	CISTERNAL PROCEDURE			TOTAL NO. OF DOGS	DEGREE OF DEPRESSION*					RECTAL TEMP., RISE OF	
	Vol. c. s. f. withdrawn	Reinjected	Vol.		0	1+	2+	3+	4+	Range	Average
			cc.								
I	8	"Fatigue" c. s. f.	8	20	0	2	9	6†	3	1.8–4.4	2.9
II	8	Normal c. s. f.	8	12	1	5	5	1	0	1.6–4.2	2.5
III	8	Autogenous c. s. f.	8	6	0	2	3	1	0	1.4–4.0	2.5
IV	8	0.9% NaCl sol.	8	6	0	1	3	2	0	1.8–3.2	2.6
V	8	Autogenous c. s. f.	5–6	6	1	3	2	0	0	0.0–3.5	1.5
VI	0	Normal c. s. f.	3	6	0	2	2	2	0	2.2–3.7	2.7
VII	8		0	6	6	0	0	0	0	0.0–0.8	0.3
VIII	7	Air	7	6	0	5	1	0	0	1.4–3.0	2.2
IX	5	Lateral ventricle "fatigue" c. s. f.	5	2	0	2	0	0	0	2.2–2.5	2.3

* See methods for the definition of 1, 2, 3, 4 plus.

† c. s. f. Pressure at height of depression 22.0, 44.0 cm. and 36.4 cm.

manifests nodding; when his eyes close his head droops and his legs weaken as if he were about to sink to the floor. He suddenly awakes with a start. After about two hours the animal is soundly "asleep," as semi-comatose as a healthy 3 year old child two hours after falling asleep. The animals begin to awake from 3 to 4 hours after the injection and are entirely normal at from five to eight hours and remain so thereafter.

Only three of twenty dogs which received "fatigue" cerebrospinal fluid (table 1) manifested a 4 plus depression, i.e., we observed the same variability in response reported by Pieron. There was no significant correlation between the time in days necessary to produce fatigue in the donor of "fatigue fluid" and the degree of depression that resulted in the recipient. The donors of the three recipients which showed a 4 plus depression had

been kept awake for 9, 11, and 13 days. The average time that the 20 "fatigue" dogs were kept awake was 12 days; the minimum was 7 and the maximum 16 days.

The respiration was quiet and regular throughout the period of depression. In the three dogs which manifested a 4 plus depression the respiration was slowed, regardless of a rise in body temperature. In the others the respiration tended to be slower than normal. In three when their body temperature became elevated to about 105°F., panting occurred.

Coincident with the onset of depression the rectal temperature rose, reaching a peak at from two to five hours after the injection. Six to eight

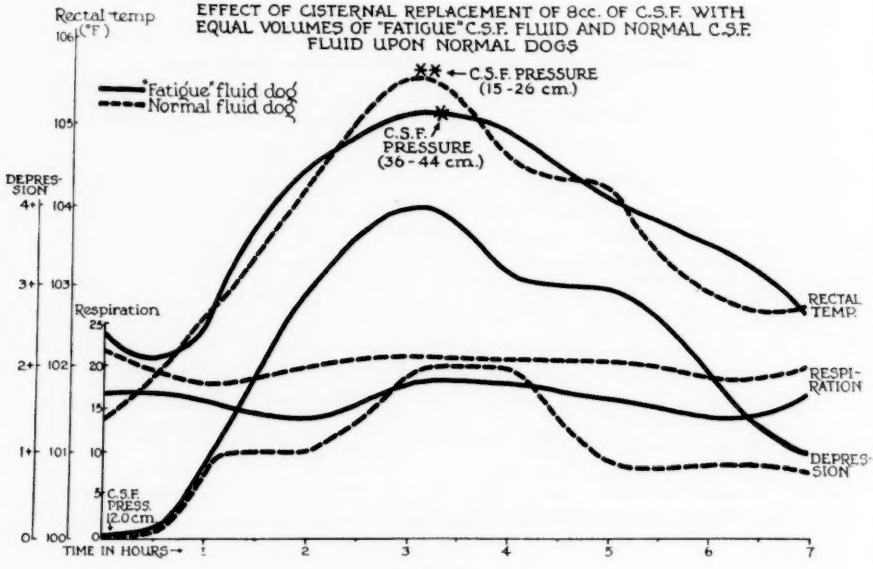
EFFECT OF CISTERNAL REPLACEMENT OF 8cc. OF C.S.F. WITH EQUAL VOLUMES OF "FATIGUE" C.S.F. FLUID AND NORMAL C.S.F. FLUID UPON NORMAL DOGS
Rectal temp (°F)
"Fatigue" fluid dog
Normal fluid dog
C.S.F. PRESSURE (15-26 cm.)
C.S.F. PRESSURE (36-44 cm.)
DEPRESSION
Respiration
RECTAL TEMP.
RESPIRATION
DEPRESSION
C.S.F. PRESS. 12.0 cm.
TIME IN HOURS

Fig. 1

hours after the injection the temperature was normal. A strict correlation between the curve of body temperature and the curve of depression in each dog did not occur. When these results were averaged, however, a close correlation was found (fig. 1).

In two of the 20 dogs which received "fatigue" fluid some evidence of catalepsy was noted, or they appeared as if they had been given a small dose of bulbocapnine. These two dogs showed later a 3 plus depression. A few of the dogs shivered as the temperature rose and manifested some muscular spasticity. Some of the dogs which did not show a 4 plus depression, appeared to have a stiff neck. The stiff neck did not result

from the needle, because inserting a needle without withdrawing fluid, or on withdrawing and not replacing fluid, did not cause the neck to appear stiff.

Group II. *The effect of withdrawal of 8 cc. of fluid and its replacement with 8 cc. of fluid from a normal animal. Control experiment.* To ascertain whether the rise in temperature and the depression observed was due to a reaction to the homologous cerebrospinal fluid injected, or to "hypnotoxin," we removed 8 cc. of fluid from a normal dog and replaced it with 8 cc. of fluid from another normal dog. Twelve such experiments were performed. The animals were depressed, but not to the degree as those receiving "fatigue" cerebrospinal fluid (table 1). The temperature was elevated in this control group on the average to the same extent as those in the group receiving "fatigue" fluid. (Table 1 and fig. 1.) The other symptoms were the same as observed in the animals of group I except none manifested catalepsy. As in group I, immediately and for about 20 minutes after the injection, the animals were normal in every respect.

Groups III and IV. *An autogenous controlled injection of cerebrospinal fluid.* Is the depression and temperature rise due to a reaction to homologous cerebrospinal fluid? It will be noted from the results in table 1 that the degree of depression and temperature rise was about the same in both these groups as in the controls of group II. Thus, the depression and temperature rise was not due to a reaction from the transfer of cerebrospinal fluid from one dog to another. It is evident that a temporary alteration in the volume of cerebrospinal fluid leads to depression and a rise in body temperature.

Groups V, VI, VII, and VIII. Cisternal puncture without withdrawing or injecting anything, and leaving the needle in place for six minutes had no effect.

The results of these experiments show *a*, that a temporary diminution of the cerebrospinal fluid volume even though returned to the original within six minutes causes varying degrees of depression and a rise in body temperature; *b*, that the simple withdrawal of cerebrospinal fluid has no effect; *c*, that a slight increase in cerebrospinal fluid volume causes moderate depression and a rise in body temperature, and *d*, that the replacement of cerebrospinal fluid with air causes slight depression with the usual rise in body temperature. Apparently the only two operations which may be performed via the cistern without disturbing the dog are to withdraw fluid and to insert a needle.

The effect of intraventricular intervention on intraventricular pressure. When this work was initiated there was no reason to believe that the simple removal and replacement of cerebrospinal fluid in the dog would cause an alteration in cerebrospinal fluid pressure. The results of the foregoing experiments, however, raised the question.

The effect of withdrawing and replacing 8 cc. of cerebrospinal fluid on intraventricular pressure was determined in seven dogs under pentobarbital anesthesia. A needle was inserted into the cisterna and connected to a manometer (1 mm. bore) so as to prevent a flow of fluid into or out of the cisterna (4). After the initial pressure was measured 8 cc. of cerebrospinal fluid were withdrawn and replaced during 6 minutes. The cerebrospinal fluid pressure was then measured and the temperature recorded at intervals over a period of from 6.0 to 7.0 hours. Six pentobarbitalized dogs were used as controls; no fluid was withdrawn and replaced (5).

The results are shown in table 2. The half-hour readings are averaged and graphed in figure 2. It is evident that the simple withdrawal and

TABLE 2

Effect of intraventricular intervention upon intraventricular pressure and rectal temperature in the anesthetized dog (nembutal)

DOG	CISTERNAL WITHDRAWAL AND REPLACEMENT OF 8 CC. C. S. F.					NORMAL CONTROLS; NO FLUID WITHDRAWN OR REPLACED				
	Basal		Peak of rise			Basal		Peak of rise		
	Press.	R. T.	Press.	Temp.	Time	Press.	R. T.	Press.	Temp.	Time
	cm.	*°F.*	*cm.*	*°F.*	*hr.*	*cm.*	*°F.*	*cm.*	*°F.*	*hr.*
1	11.6	101.0	42.5	102.2	4	9.5	100.8	18.2	100.5	4
2	10.9	101.7	37.5	104.6	3	10.0	100.6	16.8	96.8	5.5
3	10.0	102.6	26.2	104.7	4.5	13.8	101.3	20.0	103.0	5.5
4	11.5	102.3	53.2	105.5	5.5	8.0	102.6	13.4	101.8	4.5
5	10.2	101.8	56.0	102.4	1.3	9.1	99.5	15.6	101.5	5
6	9.5	100.7	33.0	102.4	3.5	8.5	103.6	17.2	105.8	8.5
7	10.3	100.2	48.0	102.6	5.3					
Av.....	10.5	101.4	42.3	103.5		9.8	101.5	16.8	101.8	

replacement of 8 cc. of cerebrospinal fluid causes a rise in intracranial pressure and body temperature. A significant change in rate of respiration did not occur. The intracranial pressure rose only slightly in the control animals.

In view of this evidence, we measured the intracranial pressure via the cisterna in three dogs in which 8 cc. of fluid were withdrawn and replaced with 8 cc. of "fatigue" cerebrospinal fluid. The intracranial pressure was measured in the animals at the height of the depression, when the measurement could be easily and accurately made because of the depressed state of the animal. In one animal the depression was 2 plus and the cerebrospinal fluid pressure was 22.0 cm. In three the depression was 3 plus and the pressure was 44.0 cm., 41.5 cm. and 36.4 cm.

In several other experiments similar to the foregoing, we confirmed the

fact that the intravenous injection of hypertonic glucose solution (50 cc. of a 50 per cent solution) would decrease intraventricular pressure.

Further experiments with the c. s. f. from fatigued dogs. Since in the controls of group V (table 1) only a slight rise in temperature resulted when 8 cc. of fluid were withdrawn and replaced with only 5 or 6 cc., we decided to repeat this experiment with "fatigue" fluid. In three dogs 8 cc. of fluid were withdrawn and replaced by 5 cc. of "fatigue" fluid. A 2 plus depression occurred in two of the three animals and a three plus in the third. The average temperature rise amounted to 2.5°F.

Four dogs (not included in table 1) which had received "fatigue" fluid were given 50 cc. of a 50 per cent solution of glucose at the point we considered to be the height of their depression. An apparent, definite

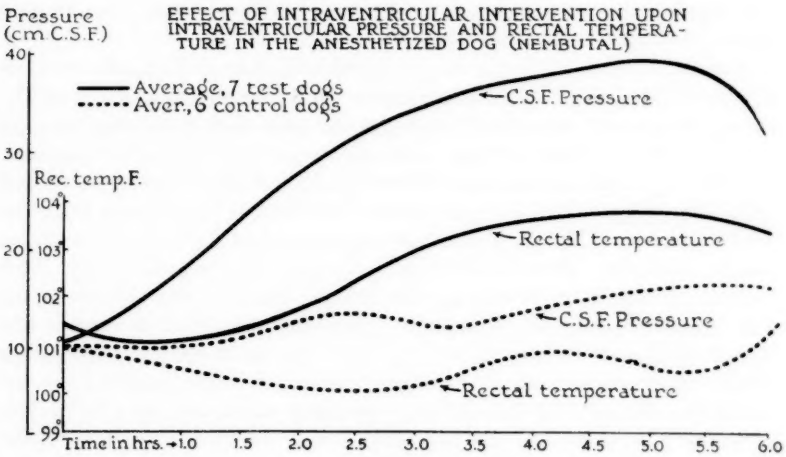

Fig. 2

improvement in the depression occurred for a period of about 30 minutes after the completion of the injection. The simple insertion of a needle into a vein did not produce the effect. The elevated body temperature of these animals was not influenced by the injection of glucose.

Injection of "fatigue" fluid into the lateral ventricle. The parietal bone of five dogs under anesthesia was trephined so that the lateral ventricle could be reached by a needle after healing of the wound several weeks later.

Five cubic centimeters of c. s. f. were withdrawn and replaced with 5 cc. of "fatigue" fluid in two of the dogs (table 1). The usual elevation of body temperature occurred but the depression was slight.

In the three other trephined animals a needle was inserted into the

cisterna and another into the lateral ventricle. As fluid was withdrawn from the cisterna an equal quantity of "fatigue" fluid was injected into the ventricle. One of the dogs showed a 1 plus and the others a 2 plus depression. The body temperature was elevated 1.7°F.

We attempted to use the lumbar region as an exit for the drainage of the c. s. f., but we were never able to obtain more than 2 cc. of fluid from that region.

"Fatigue" fluid administered by the vascular route. The intravenous injection of 14 cc. of "fatigue" fluid into 3 dogs had no effect. Sixteen cubic centimeters of "fatigue" fluid was injected into the carotid artery of normal dogs, the carotid being exposed under novocaine. No effects were observed.

One hundred cubic centimeters of whole blood (dogs were tested for compatibility) were transfused from three "fatigued" dogs into normal dogs. No effects were observed.

OTHER STUDIES. *Effect of encephalography in man.* Through the courtesy of Dr. Loyal Davis we were able to study the records and to observe directly in several instances the effect of the procedure of encephalography on 61 patients. None of these patients received a sedative or headache powder. In these patients from 30 to 200 cc. of fluid were withdrawn and from 20 to 190 cc. of air were injected. The average rise in body temperature amounted to 2.3°F. and ranged from 0 to 3.6°F. Usually a severe headache appears when from 50 to 75 cc. of air have been injected. The headache persists for from 12 to 24 hours. Some of the patients retch and about one-half are nauseated. (This did not occur in any of our dogs.) Pallor of the skin and perspiration occur frequently. An initial fall in body temperature occurred in nine patients and ranged from 1.0 to 1.8°F. The peak of the rise in temperature occurred from 4 to 8 hours after the procedure and the temperature was elevated above normal for from 8 to 24 hours. The c. s. f. pressure (lumbar) in these patients initially ranged from 350 to 550 mm. H_2O in the sitting position; it was not determined at the height of the temperature rise. Because of the headache these patients, as a rule, remain very quiet in bed. They say that they do not sleep because of the headache; they "feel sleepy but can't sleep, but may doze a little."

The rise in body temperature is the only point of similarity objectively determined between these patients and our animals. We obtained a similar rise in temperature in the normal dogs when we removed 8 cc. of fluid and replaced it with 7 cc. of air. Our dogs were only slightly depressed.

The effect of fever produced by dinitrophenol. Fever was produced in three dogs by the intramuscular injection of dinitrophenol. No points

of similarity between the behavior of our dogs subjected to intraventricular interventions and the dogs with fever due to dinitrophenol were observed, except that panting occurred in a few of the former dogs and everytime in the latter. The dinitrophenol fever did not significantly alter the c. s. f. pressure in two dogs.

Relation of the liver to sleep in white mice. H. Holmgren (6) observed that an aqueous extract of the liver of a sleeping (day) mouse had an hypnotic effect when injected subcutaneously (1–2 cc.). The extract of the liver of an active (night) mouse had no such effect.

We have repeated Holmgren's experiment in detail observing 12 white mice. Ten received the injections of the extracts; two received normal saline injections. We observed no difference in the nocturnal activity of the mice receiving either the "day" or "night" liver extracts and the normal saline controls. We were surprised to find that the extract did not apparently sicken the mice; we did not compare food intake with and without the liver extracts, however.

The effect of pituitrin injected into the cisterna. Pituitrin was injected intraventricularly (7) because some have thought that the secretion of the posterior lobe of the hypophysis passes into the c. s. f. The first dog received 0.1 cc. of surgical pituitrin into the cistern (1 cc. of c. s. f. was removed and 0.1 cc. of pituitrin was injected) at 2:30 p.m. No effect was noted. At 4:30 the dog was again given 0.2 cc. Prostration, defecation, apnea followed by tachypnea occurred promptly. At 4:35, tachypnea was still present, the dog could sit up but not stand, was asthenic, but alert. At 5:00 the dog was still asthenic but alert and wagging tail. Fifteen one hundredths cubic centimeters of pituitrin was injected into two other dogs and had no significant effect. A fourth dog was given 0.2 cc. of pitressin (Parke, Davis & Co.) into the cisterna. Immediately after the injection the dog was normal, but rubbed his nose as if it itched, and hiccoughed for several minutes. During the next 20 minutes the dog preferred to lie on his side (some asthenia), but was awake, alert and playful.

The effect of acetylcholine injection into the cisterna magna. Dikshit (8) reported the production of "a condition closely resembling sleep" by the injection of small doses (0.1–0.5 gamma) of acetylcholine into the lateral ventricle or deeper into the hypothalamus in previously trephined cats. The effect appeared in from 10 to 30 minutes after the injection and lasted for from 2 to 3 hours. He states that control injections of normal saline produced no definite effect.

In two normal dogs 4.0 cc. of c. s. f. were withdrawn from the cistern and choline HCl, 6.0 mgm. in one case and 8.0 mgm. in the other, was dissolved in it and the fluid was slowly reinjected. No effect was observed

in either dog (body temperature was not followed). Marinesco (9) reported that "sleep" occurred after a preliminary period of agitation and hypertonus when choline was injected into the third ventricle.

The results of A. C. injection into the cisterna and lateral ventricle are summarized in table 3. The dogs 1, 2, and 3, which received the larger doses of a. c., vomited, defecated and urinated promptly and then from 10 to 30 minutes after the injection manifested asthenia of the limbs out of proportion to the degree of cerebral depression. The two dogs receiving 8 and 16 gamma of a. c. into the lateral ventricle manifested

TABLE 3

Effect of ventricular injection of acetylcholine on the dog

DOG NO.	ROUTE OF INJECTION	FLUID WITHDRAWN	FLUID REPLACED	DOSE OF A. C.	DEGREE OF DEPRESSION	RECTAL TEMP. RISE	REMARKS
		cc.	cc.			°F.	
1	Cistern	4.0	1.5	1.5 mgm.	+		Asthenia, awake, could not stand
2	Cistern	4.0	1.5	1.0 mgm.	+		Asthenia, awake, could not stand
3	Cistern	4.0	2.0	3.0	+		Asthenia, awake, could not stand
4	Cistern	0.75	0.1	4γ	+	0.6	Weakness in hind legs
5	Cistern	0.75	0.2	8γ	++	3.1	Weakness in hind legs
6	Cistern	1.0	1.0	2γ	0	0	No effect
7	Cistern	1.0	1.0	4γ	0	0.8	No effect
8	Cistern	1.0	1.0	6γ	+	0.4	No effect
9	Lat. Ventr.	1.0	0.2	8γ	+++	1.0	Dog sways on being stood up, then lies down and closes eyes as in sleep
10	Lat. Ventr.	1.0	0.4	16γ	+++	4.0	Dog sways on being stood up, then lies down and closes eyes as in sleep

what we call a typical depression or "sleep reaction"; the dog that received 16 gamma, however, vomited and defecated within a few minutes after the injection. As reported by Dikshit (8) a condition resembling sleep was observed particularly in the two dogs receiving a. c. into the lateral ventricle. The temperature elevation, however, vitiates the physiologic value of the response.

The effect of injection of a. c. into the lateral ventricle on c. s. f. pressure as measured in the cisterna magna was determined in three dogs anesthetized with pentobarbital. In the first dog the original body temperature was 99.2°F.; the c. s. f. pressure during a control period of one hour varied

from 10.0 to 10.5 cm., during which period the body temperature fell to 97.6°F. One cubic centimeter of fluid was withdrawn from the lateral ventricle and replaced with 0.8 cc. of sodium chloride solution containing 16 gamma of a. c. In fifteen minutes the pressure rose to 21.0 cm. and fell during the course of an hour to 12.2 cm., the body temperature at this time being 96.2°F. Then 16 gamma more of a. c. were injected and the c. s. f. pressure rose to 41.0 cm. in 1.25 hours, the body temperature being 97.6°F., after which the pressure fell to 24.6 during the course of 2.5 hours. In a second animal the pressure was not elevated by the first injection and the body temperature did not change. After the second injection the pressure rose to 30.3 cm. and the body temperature from 102°F. to 104.8°F. in 2.5 hours, after which both returned toward normal. In the third dog after 16 gamma of a. c., the pressure rose from 12.0 cm. to 44 cm. and the body temperature was elevated 2.8°F.

Is acetylcholine present in the cerebrospinal fluid of normal and fatigued dogs? We did not multiply experiments of the foregoing type because of the pathological nature of the depression or "sleep" observed. We considered it more pertinent to make assays for a. c. in the c. s. f. of fatigued dogs.

We obtained 3 cc. samples of c. s. f. (0.2–0.4 mgm. eserine) by cisternal puncture from 5 fatigued (7–14 days) unanesthetized dogs, and assayed it within one minute for a. c. using the eserinized rectus abdominis of the frog (10), which reacted to 0.05 gamma per cubic centimeter of a. c. in our hands. The dorsal muscle of the eserinized leech (Hirudo medicinalis) was also used, which detected 0.003 per cc. in our hands. We could not detect a. c. in the cerebrospinal fluid of the fatigued dogs. We also assayed the cerebrospinal fluid of normal unanesthetised dogs for a. c. and found none.

The negative results prompted us to repeat the work of Dikshit (8) and of Feldberg and Schriever (12), who had under certain experimental conditions found a. c. in the cerebrospinal fluid.

Samples (2–3 cc.) of cerebrospinal fluid were removed from 18 dogs under pentobarbital and chloralose (1 dog) anesthesia, before, immediately and 15 minutes after slow (20–30 min.) intravenous injection of eserine salicylate in doses varying from 1.5 to 5.0 mgm. per kilo. The dogs then each received another 1 mgm. of eserine followed by 2 or 3 cc. of adrenalin (1–1,000) or by 5 minutes of asphyxia, or by interrupted stimulation of the central end of the vagus (10–20 min.) with a current sufficient to cause a cessation of respiration. Samples of cerebrospinal fluid (2–3 cc.) were withdrawn and immediately assayed after each procedure in a 10 to 50 per cent dilution. To prevent the respiratory and cardiac effects of the large dose of eserine, each dog received 2 mgm. of atropine sulphate intravenously.

Our results will not be recorded in detail because they agreed with those of Feldberg and Schriever (12), except that we could detect no acetylcholine in the cerebrospinal fluid assayed in high dilution (10–15 per cent) or when quantities of eserine below 3.0 mgm. per kilo were used.

The foregoing results certainly rule out acetylcholine as being a possible "hypnotic" substance in the cerebrospinal fluid of fatigued dogs. The results throw no light on whether a. c. is produced by synapses in the brain. If a. c. passes into the cerebrospinal fluid, it should not be destroyed, because Stedman and Stedman (13) failed to find an esterase in cerebrospinal fluid. Yet, Nochrinowski (14) has reported a. c. in the spinal fluid of patients afflicted with certain diseases of the central nervous system. If a. c. is produced by central synapses, it must be destroyed by an esterase in the brain substance; and if it passes into the cerebrospinal fluid, there must be a deficiency in brain esterase action. Such a deficiency is not present in fatigued dogs because we failed to find a. c. in their cerebrospinal fluid. We did not inject eserine into the ventricles because we were primarily interested in ascertaining whether a. c. was the substance in fatigue fluid which causes the more marked depression when this fluid is injected. It is remotely possible that a reaction product of a. c. and cholinesterase may be concerned.

Discussion. We are convinced that we have confirmed the occurrence of the phenomenon observed by Pieron (1). We are also convinced that the cerebrospinal fluid from fatigued dogs produces a greater depression of the central nervous system than the cerebrospinal fluid from normal dogs. But the complete picture of normal sleep does not occur after the replacement of 8 cc. of the cerebrospinal fluid from a fatigued dog. The most certain difference between normal sleep and the "depression" we observed with the fatigue fluid was the rise in body temperature, which is known not to occur in normal sleep (15, 16, 17).

It is certain that an increase in the brain volume of trephined subjects occurs in sleep (18–19). But whether the increase in brain volume, if it occurs in the intact subject, is actually accompanied by an increase in intracranial pressure is uncertain. It is known that intracranial pressure may rise to such heights as to induce coma; on the other hand it is known clinically that the cerebrospinal fluid pressure can be definitely elevated (55.0 cm.) without concomitant depression or sleep resulting. That increased intracranial pressure is not requisite for the onset of sleep is crucially demonstrated by the fact that 8 cc. of cerebrospinal fluid (at least one-third the total volume) may be withdrawn from a fatigued dog and he will be profoundly asleep five minutes later. On the other hand, the intravenous injection of hypertonic glucose, which is known to diminish temporarily intracranial pressure, into dogs showing a three plus depression decreases the degree of depression for a period of about thirty minutes.

Although this evidence suggests that the increase in intracranial pressure, observed in the dogs following the withdrawal and replacement of equal quantities of cerebrospinal fluid, contributes to the depression, it is not direct proof. We know that the afferent sensations caused by the insertion of the needle into the vein were not concerned, but other sensations arising from the hypertonic glucose may have been concerned in awakening and keeping the animal partially or more awake for a period.

It may be that the rise in body temperature plus the rise in intracranial pressure was the cause of the depression or "sleep." We did not measure the intraventricular pressure in a sufficient number of the control animals and the animals which received the "fatigue" cerebrospinal fluid to answer the question. The rise in body temperature is not correlated with the degree of depression. This question was not investigated further because: *a*, we would have had to increase the animals in our series so as to obtain data of statistical significance; and *b*, we had no strict quantitative method for determining the degree of depression. We attempted to devise a quantitative method for the purpose by ascertaining the intensity of a sound which would cause the dog to open his eyes a fixed length of time after they were spontaneously closed. This method was only of qualitative value.

That a rise in body temperature and cerebrospinal fluid pressure and pleocytosis of the fluid may occur after encephalography, removal and reinjection of spinal fluid, and other lumbar and ventricular interventions is reported in the clinical literature (20–31). The temporary alteration in the volume of fluid and the c. s. f. p. must mildly traumatize the capillaries of the brain or choroid plexus.

Though the Pieron phenomenon may be due entirely to a disturbance of intracranial pressure and body temperature regulation, our observations do not permit such a conclusion. We suspect that a detailed examination of the chemistry of the cerebrospinal fluid of the fatigued dog will reveal differences from the cerebrospinal fluid of the normal dog, which will account for the greater degree of depression observed with the fatigue fluid. We believe that the change or changes in "fatigue" cerebrospinal fluid, if they are found, will only be a reflection of the underlying chemical changes in the brain conducive to fatigue or sleep. It was for this reason that the study of a. c. in the cerebrospinal fluid was undertaken. Although the results of the study show that a. c. is not present in the cerebrospinal fluid of fatigued dogs, the possibility that the acetylcholine mechanism may not be concerned has not been entirely ruled out. In this connection the observations of Henderson and Wilson (32) on human subjects are pertinent. They introduced very large doses of a. c. into the lateral ventricle of patients prepared for ventriculography. Deep sleep resulted in one and light sleep in two, the remaining five showed no tendency toward

sleep. The rectal temperature did not rise probably because of perspiration, which does not occur in dogs. The most uniform symptoms produced were nausea, vomiting and intestinal peristalsis. Intraventricular pressure changes were not reported. The variability of the depression observed in these human studies, as well as in the studies on animals, may be explained in a number of ways, e.g., dosage, visceral stimulation keeps the animal awake, etc. One could propose at least two speculative hypotheses for the cause of sleep on the basis of the concept of the chemical transmission of nerve impulses; but such speculation is probably not warranted in view of the paucity of directly pertinent facts.

SUMMARY AND CONCLUSIONS

1. Nine of twenty dogs in which 8 cc. of cerebrospinal fluid were withdrawn during 3 minutes and then replaced by an equal quantity of cerebrospinal fluid from dogs kept awake in the standing position for from 7 to 16 days showed a depression of the central nervous system or "sleep" which was graded either 3 or 4 plus. Only four of twenty-four control dogs which received in the same manner either fluid from a normal dog or their own fluid or normal saline solution showed a 3 plus depression and none a 4 plus depression. To this extent the observations of Pieron have been confirmed. *The normal picture of sleep is not produced* because the body temperature of both groups of animals is elevated approximately 2.6°F. Further, the cerebrospinal fluid pressure is elevated, the maximum elevation being to approximately 44 cm. of normal saline solution.

2. The simple withdrawal and reinjection (warmed syringe) of 8 cc. cerebrospinal fluid during a period of six minutes causes a rise in ventricular pressure and body temperature in anesthetised dogs.

3. The elevated intracranial pressure is probably a factor which operates to augment the depression that occurs after the cisternal injection of cerebrospinal fluid from a fatigued dog, because the intravenous injection of hypertonic glucose decreases the degree of depression for a 20 or 30 minute period.

4. A rise in intracranial pressure cannot be a feature of prime importance in causing the onset of sleep because when 8 cc. of cerebrospinal fluid are withdrawn from a fatigued dog, such a dog will fall into a profound sleep within five minutes. The withdrawal of a similar quantity of fluid from a non-fatigued dog does not cause sleep or objective changes.

5. Our results indicate that a detailed assay of the cerebrospinal fluid of fatigued dogs *may* yield results which will reflect the underlying chemical changes which lead to fatigue and are conducive to sleep.

6. Acetylcholine could not be detected in the cerebrospinal fluid of normal or fatigued dogs. The possibility that fatigue interferes with the "chemical mechanism for the transmission of nerve impulses" has not been ruled out by the results of our study of the acetylcholine mechanism.

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THE EXCRETION OF SULFATES BY THE DOG

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Among the first studies inspired by Cushny's theory of renal function was the one undertaken by Mayrs, who investigated the excretion of sulfates as compared with the elimination of a number of other substances. He injected sodium sulfate intravenously into rabbits in order to raise the concentration of sulfate in the plasma to allow its determination with a sufficient degree of accuracy and found that sulfates were concentrated approximately twice as much as urea (maximum: 2.75, minimum: 1.50). In a subsequent series of experiments the degree of concentration of sulfates ("concentration ratios") was compared with that of phosphates and of creatinine and all these values were found to be approximately the same.

White could not find as satisfactory agreement between ratios of concentration of different substances as compared with the concentration ratio of sulfates and concluded that "no fixed rule such as Cushny sought to apply in his 'modern theory' can be assigned to the process of sulphate elimination." The same investigator, in collaboration with Monaghan, in a subsequent study of phlorhizinized dogs was led to recognize the important fact that "the sulphate clearances are always greater with high plasma sulphate levels than with normal, with rough proportionality."

In man the endogenous sulfate clearances were studied by Macy, who reported that they were 10 to 50 per cent less than those of urea. Cope found essentially analogous clearance ratios. He could not discover any influence of moderately increased sulfate levels as obtainable by the oral administration of sodium sulfate on sulfate clearance. Hayman, on the other hand, recognized a very definite tendency of the concentration ratio of sulfates to approach that of creatinine after the intravenous administration of the former.

In the last few years, thanks to the work of Richards, Smith and Shannon, and Van Slyke and co-workers along the lines of Cushny's theory, the equality of the clearances of creatinine, inulin and ferrocyanide in dogs has been firmly established. Apparently the magnitude of these clearances at the same time indicates the extent of glomerular filtration. Generalizing, it can be said that in the dog a rate of excretion in excess of simul-

taneously determined creatinine, inulin or ferrocyanide clearances is considered very widely as strongly suggestive evidence of active secretion by the tubules, whereas rates of excretion below the clearance of one such standard test substance would be indicative of tubular reabsorption.

In the study which is to follow, the excretion of sulfate has been studied in the dog under a variety of conditions and has been compared with the simultaneous excretion of injected creatinine.

PHYSIOLOGIC PROCEDURE. All dogs were between 10 and 15 kgm. in weight and they all received a standard diet consisting of 38 per cent horse meat, 35 per cent cracker meal, 15 per cent cooked tomatoes, 8 per cent lard and 4 per cent bone ash. This diet was fed in amounts calculated to be the equivalent of twice the basal caloric requirement for the individual animal. Until the beginning of each experiment water was allowed *ad libitum*. Food was withheld for periods of from seventeen to twenty-four hours preceding the experiment.

In most experiments in order to obtain a desirable volume of urine, 200 to 500 cc. of tap water were introduced by stomach tube before the beginning of the clearance periods; also, 400 to 800 cc. of a 0.64 to 0.72 per cent solution of sodium chloride was given intravenously. The beginning of the clearance periods was preceded by the intravenous injection of 1 cc. of a 10 per cent solution of creatinine per kilogram body weight. Immediately after this, administration of additional creatinine was started in order to maintain its level in the plasma. This was accomplished by the intravenous injection at a constant rate (by means of a Woodyatt pump) of approximately 200 cc. per hour of a solution containing 0.3 per cent creatinine and 5 per cent *d*-glucose ("creatinine maintenance solution"), the *d*-glucose serving the purpose of making the solution approximately isotonic to the blood.

Twenty to thirty minutes after the beginning of the injection of the "creatinine maintenance solution" the bladder was emptied. Usually twenty-five minutes was allowed for each clearance period, in the middle of which blood was drawn.

In those experiments in which the rate of excretion of sulfate was investigated at plasma concentrations higher than normal, the increased plasma level was obtained by the intravenous administration of sodium sulfate in a 10 per cent solution. When larger amounts were being given the rate of injection amounted to 5 cc. per minute. A rise of approximately 20 mgm. of sulfate (expressed as SO_4) per 100 cc. of plasma was found to result from the introduction of 100 mgm. of sodium sulfate per kilogram body weight. Maintenance of the concentration of sulfate in the plasma so reached was attained by the addition of suitable amounts of sodium sulfate to the "creatinine maintenance solution."

CHEMICAL PROCEDURES. The blood after it was drawn was mixed with

heparin in a 15 cc. graduated centrifuge tube. In those instances in which the *hematocrit value* was determined, the total amount of blood in the sample was noted and the volume of the packed erythrocytes was determined after centrifugation for two hours at a speed of 2,800 revolutions per minute and a temperature of 1.5°C.

Sulfates in plasma and urine were determined volumetrically on trichloracetic acid filtrates after precipitation with benzidine according to the method of Power and Wakefield.

Creatinine in the plasma was determined in tungstic acid filtrates; the color was developed essentially acording to the method of Folin and Wu, using an alkaline picrate solution. The resulting colors were compared with those developed in two standard solutions, using the Goudsmit-Summerson variable layer photoelectric comparison photometer as a measuring instrument.

Creatinine in the urine was determined on portions containing approximately 1 mgm., essentially according to the method of Folin, and in the manner described for the determination in the blood.

Calculation. "Clearances" throughout this study have been calculated as $\frac{\text{concentration of substance in the urine}}{\text{concentration of substance in the plasma}} \times$ volume of urine in cubic centimeters per minute. In all instances the "clearance ratio," viz., $\frac{\text{sulfate clearance}}{\text{creatinine clearance}}$, has been calculated and used in preference to the sulfate clearance itself. Creatinine clearances have been calculated without correction for "endogenous" creatinine values, since Goudsmit has shown that the "endogenous" creatinine disappears from the blood flowing through the kidney at a rate rather similar to the one ("extraction percentage") which Van Slyke, Hiller and Miller determined for exogenous creatinine. Thus the clearance of the "endogenous plus exogenous" creatinine equals that of "exogenous" creatinine alone.

Clearance ratios have been calculated to three decimal places (e.g., 0.103) because we believe that two would not do full justice to the consistency of our findings. It is realized that the third decimal place cannot be relied on to the full extent of its value. Equally the value 1.063 has its third decimal place included only for the sake of uniformity; in itself, 1.06 covers all the accuracy that can be claimed.

RESULTS. Since a substance in order to be available for glomerular filtration should be present in the plasma in a freely diffusible state, *ultrafiltration studies* were carried out on three different samples of serum. After having been placed in collodion sacks, the samples were subjected to a pressure not exceeding 30 cm. of mercury. The results, seen in table 1, clearly indicate that most of the sulfate present in dog's serum, if not all, is ultrafiltrable through a collodion membrane. This is in agreement

with the observations of Hayman on human blood serum and, together with the observation that sucrose and sulfate, after their intravenous injection, are distributed in the same fashion throughout the fluids in the body, (12, 15) meet, in great part at least, the statement of Peters: "Until the permeability of vascular and cellular membranes to sulfates has been determined, it is futile to attempt an analysis of the mode of excretion of this ion."

Sulfate clearance at normal (endogenous) concentrations of sulfate in the plasma. A total of twenty-nine clearance periods have been available for analysis. They were obtained on six dogs, to none of which sulfate had been administered in any form (except for whatever small amount was

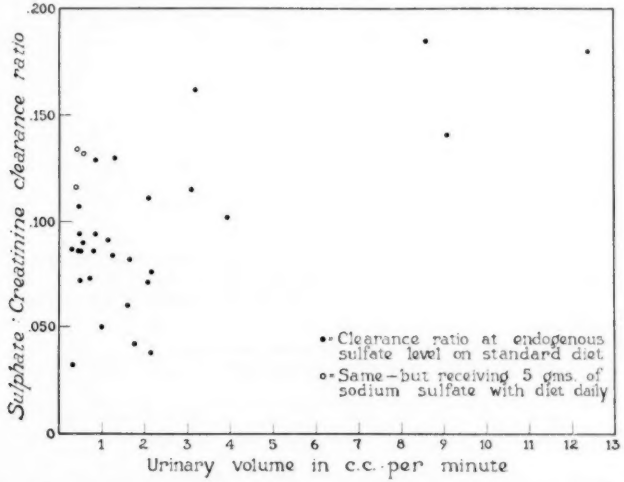

Fig. 1. Sulfate: creatinine clearance ratios at endogenous levels of sulfate in the plasma and their relationship to urinary volume.

contained in their diet) for a period of at least two weeks preceding the experiment.

The normal concentration of sulfate in the plasma of dogs was found to vary between 11.5 and 18 mgm. of SO_4 per 100 cc. of plasma, most of the values falling between 12 and 15 mgm. In one instance after the administration of 1,500 cc. of water partly by the oral and partly by the intravenous route, values as low as 10.4 mgm. were recorded.

In figure 1 the clearance ratios obtained at normal plasma levels have been plotted against urine volume. As can be seen, the scattering is fairly large; still the general shape strongly suggests an increase of the clearance ratio as the extent of diuresis rises. In other words, no "aug-

mentation limit" can be demonstrated to enter into the mechanism of elimination of sulfates in the dog. A similar situation might well exist with regard to the excretion of sulfates in man, although Hayman did not choose to interpret the clearances plotted in his graphs in this manner. In this connection it seems worthy of note that Shannon found in dogs a progressive increase of the ratio of urea clearance to creatinine clearance with increased urinary volume without its ever reaching a point which could be termed an augmentation limit. Recently figures have been presented indicating that in man, too, "there is a progressive increase in the urea clearance as the urine flow increases from low to high values" (4).

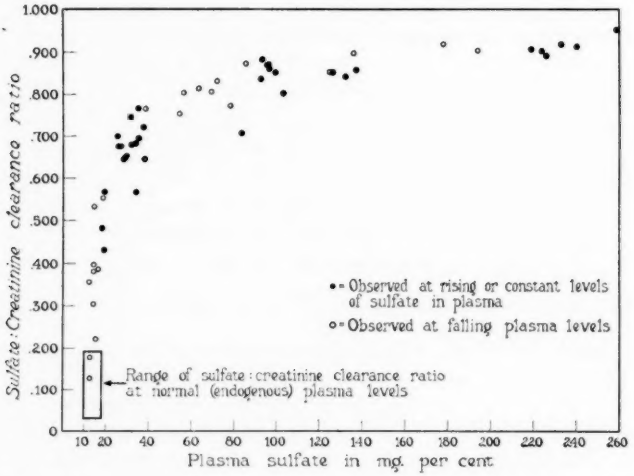

Fig. 2. Sulfate: creatinine clearance ratios at increased concentration of sulfate in the plasma.

Sulfate clearance at increased concentrations of sulfate in the plasma. In figure 2 fifty-six individually observed ratios of sulfate clearance to creatinine clearance obtained in eight different experiments on three different dogs have been plotted against the concentration of sulfates in the plasma. Details about an illustrative individual experiment and the actual results on the analytic data obtained, as well as clearance ratios calculated from those data, are recorded in tables 2 and 3. As can be seen, SO_4 concentrations as high as 259 mgm. per 100 cc. of plasma have been reached and a total of six clearance periods at levels of over 215 mgm. have been included. At these high levels sulfate clearance ranges between 89 and 95 per cent of the creatinine clearance. At lower plasma levels the ratio becomes slightly smaller; however, at levels between 30 and 40 mgm. of SO_4 per

100 cc. of plasma the clearance ratio ranges between 56 and 76 per cent, the average being very close to 70 per cent. It is apparent that between the concentrations of SO_4 in the plasma of 30 and 259 mgm. per 100 cc.

TABLE 1

Concentrations of sulfate in dog's serum and in its ultrafiltrate

EXPERIMENT	SULFATE, MGM. PER 100 CC.			CHLORIDE, MGM. PER 100 CC.		
	Serum	Ultrafiltrate	Residue	Serum	Ultrafiltrate	Residue
1	12.7	11.78	14.8	375	365	294
	12.8		12.5			
2	13.2	14.9	11.3	374	398	324
	13.3	14.1	10.9			
3	14.6	12.9	11.7	390	393	347
	14.7	12.7	11.6	391		

TABLE 2

Illustrative protocol

Experiment 2. Dog T359, ♂, weight 12.4 kgm. Date 1/24/38

10:25 a.m.	500 cc. 0.8 per cent solution of NaCl by stomach tube.
10:39	Intravenous administration of solution A (see below) started at a rate of 200 cc. per hour.
10:51–10:53	12.4 cc. 10 per cent solution of creatinine intravenously.
11:15 a.m.–12:55 p.m.	Clearance periods 1 to 4 inclusive.
1 to 3	Solution B substituted for solution A; intravenous infusion continued at same rate.
1:10 to 1:35	Intravenous administration of 125 cc. of 10 per cent solution of Na_2SO_4 (in addition to continuous injection of solution B).
1:23	Dog vomited approximately 100 cc. of greenish-yellow fluid.
1:46 to 3	Clearance periods 5 to 7 inclusive.
3	Intravenous administration of solution A substituted for that of solution B.
3 to 5:05	Clearance periods 8 to 12 inclusive.

Solution A ("Creatinine maintenance solution")		*Solution B*	
Glucose	50 gm.	Glucose	25 gm.
Creatinine	3 gm.	Creatinine	1.5 gm.
Distilled water to make 1000 cc.		Sodium sulfate	18 gm.
		Distilled water to make 500 cc.	

of plasma no consistent differences are seen between clearance ratios obtained at rising and those obtained at falling concentrations of sulfate in the plasma (indicated by different symbols).

The question could be raised here whether the fact that sodium sulfate

acts as a diuretic is not responsible for these high clearance ratios. However, at normal concentrations of sulfate in the plasma, even higher urinary volumes were observed without the clearance ratio rising to more than a fourth the ratios obtained at high plasma levels. In addition, in no instance did we find a significant change of creatinine clearance attributable to the injection of these large amounts of sodium sulfate. Finally, bringing out the point that this increase of clearance with concentration is not a generally accepted pattern of excretion of end products of metabolism: an increase in the concentration of urea in the blood does not increase its clearance to any appreciable extent, no greater certainly than could be

TABLE 3

Detailed results of experiment described in table 2

PERIOD	TIME	URINE					BLOOD					CLEARANCES			CLEARANCE RATIO	
		Vol.	Creatinine	SO_4	Urea N	Cl	Hematocrit	Creatinine	SO_4	Urea N	Cl	Creatinine	SO_4	Urea	SO_4: creatinine	Urea: creatinine
	min.	*cc. per min.*	*mgm. per min.*	*mgm. per min.*	*mgm. per min.*	*mgm. per min.*	*R. B. C. per cent*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*cc. per min.*	*cc. per min.*	*cc. per min.*		
1	25	0.48	10.85	0.632	3.34		46.7	19.5	13.7	11.2	383	55.6	4.61	29.8	0.083	0.536
2	25	0.80	9.05	0.608	2.63		48.5	18.8	14.6	11.3	382	48.1	4.16	23.3	0.086	0.484
3	25	0.84	8.58	0.572	2.57		46.9	18.2	12.9	11.6	382	47.1	4.43	22.2	0.094	0.471
4	25	1.24	9.73	0.712	3.39		44.5	18.8	16.4	10.4	382	51.8	4.34	32.6	0.084	0.629
	51	8.92	9.01		3.41	7.75		19.35*				46.6*				
5	24	10.58	10.19	128.0	3.52	9.54	39.0	19.9	259.0	8.6	349	51.2	49.4	40.9	0.965	0.799
6	25	8.24	10.43	111.2	3.14	5.84	41.1	20.5	240.6	9.35	348	50.9	46.2	33.6	0.908	0.660
7	25	7.00	9.84	99.8	3.04	4.48	42.7	20.8	226.0	8.6	357	47.3	44.2	35.3	0.934	0.746
8	25	5.48	9.96	81.8	2.82	3.00	43.3	21.3	194.0	9.75	366	46.8	42.2	28.9	0.902	0.618
9	25	3.68	9.49	54.8	2.53	1.92	46.7	21.1	135.7	8.4	380	45.0	40.4	30.1	0.898	0.669
10	25	2.40	9.15	37.9	2.36		47.5	20.2	96.1	7.7	383	45.3	39.4	30.6	0.870	0.675
11	25	1.76	9.00	27.6	2.17		48.2	19.8	78.6	7.35	383	45.4	35.1	29.5	0.773	0.650
12	25	1.36	9.22	20.9			45.8	20.1	56.7		383	45.9	36.9		0.804	

* Interpolated.

attributed to the increased urinary volume (cf. also Addis and Drury; Drury).

Retardation of readjustment of clearance of sulfate after its intravenous administration. From 30 mgm. of SO_4 per 100 cc. of plasma on downward there is a sharp drop of the ratio of sulfate clearance to creatinine clearance down to the range recorded as normal. However, a complicating factor here enters the picture, viz., that—in contradistinction to what happens at higher concentrations, as previously mentioned—the sulfate clearance ratio with falling plasma concentrations tends to be higher than that observed at rising concentrations (the actual concentration being the same in both cases).

This point was investigated further in two dogs in which the ratios of sulfate clearance to creatinine clearance were determined before and after the injection of sufficient sodium sulfate to raise the plasma level to approximately 30 mgm. per 100 cc. and the clearance ratios to approximately 0.70. The results of these experiments are shown in figure 3 and appear to indicate 1, that the ratio of sulfate clearance to creatinine clearance (the creatinine clearance remained essentially constant) does not return to the

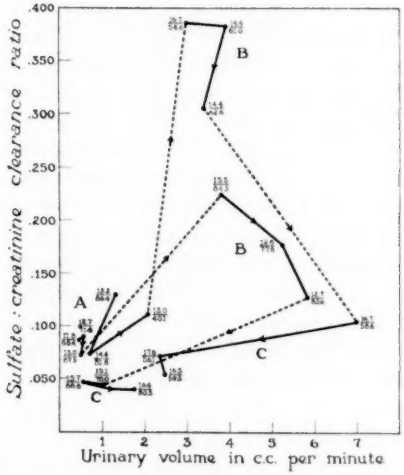

Fig. 3. Sulfate: creatinine clearance ratios at endogenous levels of sulfate in the plasma as influenced by previous intravenous administration of small amounts of sulfate.

A. Ratios before sulfate administration. B. Ratios 1.25 to 2.5 hours after discontinuing the sulfate injection. C. Ratios 22 hours later.

Solid lines indicate that clearance periods follow one another immediately; broken lines indicate lapse of time between clearance periods (see above); arrows indicate order of sequence in which clearance periods were obtained; all periods twenty-five minutes. All clearances were obtained at endogenous levels of sulfate in the plasma. The small figures adjacent to each dot indicate the level of the plasma sulfate in milligrams per 100 cc. (top) and the value of the creatinine clearance in cubic centimeters per minute (bottom).

control level as promptly as does the plasma sulfate, and 2, that the ratio of sulfate clearance to creatinine clearance may be even lower than the control levels when examined after a greater lapse of time after discontinuance of the sulfate injection (twenty-two hours).

Effect of extra sulfate in the diet on renal clearance. One animal was given 5 gram doses of sodium sulfate daily in his food. No influence on the concentration of sulfates in the serum was found after prolonged adminis-

tration. Figure 1 includes the results of the study of this dietary factor on the rate of excretion of sulfates. As can be seen, all three periods examined reveal a ratio of sulfate clearance to creatinine clearance exceeding any others obtained at comparable rates of urinary flow either in the same or in any other animal. Thus it would seem justifiable to conclude that a real increase was obtained. After discontinuing the extra sulfate in the diet the clearance ratio dropped back to normal.

Results of attempts to obtain concentrations of sulfate higher than 260 mgm. per 100 cc. of plasma. In view of our experiences with sulfate clearances at concentrations up to 260 mgm. per 100 cc. of plasma it seemed worth while to try to examine the excretion at still higher levels. This seemed justifiable since two animals had been able to stand concentrations of between 230 and 260 mgm. per 100 cc. quite well except for the vomiting which both of them had shown during the period of rising levels of sulfate in the blood. Thus, in a dog of 13.9 kgm., after ample fluids had been administered orally as well as by vein, three clearance periods were studied under standard conditions. An average creatinine clearance of 64.6 cc. per minute was observed; the average urinary volume amounted to 10.7 cc. per minute. Next the animal received intravenously 225 cc. of a solution of 5 per cent Na_2SO_4 and, in addition to that, was given at a constant rate of 300 cc. per hour, a solution containing 74.2 grams of Na_2SO_4, 33 grams of *d*-glucose, and 2 grams of creatinine per liter. A very abundant diuresis ensued.

After one and a half hours the animal appeared to be in a poor condition so that the injection had to be discontinued; 500 cc. of 5 per cent solution of *d*-glucose were administered during the following twenty minutes. After this the dog seemed to have recovered to some extent and the constant injection was reinstituted. Two consecutive clearance periods of ten minutes each were obtained at this stage with the following results: plasma SO_4 325 and 353 mgm. per 100 cc. respectively, sulfate clearance 67.4 and 50.9 cc. per minute and creatinine clearance 63.4 and 49.2 cc. per minute. The flow of urine amounted to 31.2 and 22.5 cc. per minute. Ratios of sulfate clearance to creatinine clearance calculated from these data amounted to 1.063 and 1.035 respectively. It is felt that this indicates very close approximation of both clearances at this level of sulfates in the plasma such as might be expected in view of the general trend of this ratio as indicated earlier in this paper and as illustrated in figure 2. It would seem erroneous, however, to assign too much value to the fact that the ratio is greater than unity, since the circumstances under which this part of the experiment was conducted were not as favorable as is considered necessary to form the basis of more far-reaching conclusions.

Five minutes after the last clearance period was concluded the animal died. Anatomically no apparent cause of death could be found. The

kidneys, which together weighed 90 grams, did not show any gross abnormalities. Microscopic study revealed a rather wide space between the capillaries of the glomeruli and Bowman's capsule. A slight degree of degeneration was visible in the tubular cells.

Chemical analysis of the blood taken twenty and ten minutes before death showed chlorides to be 314 and 315 mgm. per 100 cc. respectively (before sulfate administration, between 367 and 373 mgm.). Thus, judged by the sum total of sulfates and chlorides, the electrolyte concentration had been increased from 106.5 to 161.5 m. eq. per liter. One cannot help feeling that this upset of ionic concentration was one, possibly a very important, cause of rapid failing of the animal.

A similar experiment on another dog was equally unsuccessful in maintaining the life of the animal. One hour and fifteen minutes after the beginning of the injection of sodium sulfate (i.e., after the animal, which weighed 10.2 kgm., had received approximately 37.5 grams of Na_2SO_4) the blood contained 372 mgm. of SO_4 per 100 cc. and the chlorides had dropped to 298 mgm. Very shortly afterward, after muscular twitchings had been noted, the animal was seized by violent convulsions. It died approximately one and a half hour after the administration of sulfates had been discontinued. The blood removed at the height of the concentration of sulfate contained 56.5 more m. eq. of sulfate plus chloride per liter of plasma than at the outset of the experiment. Interestingly enough, the increase in sodium amounted to 130 mgm. per liter (equaling 56.5 m. eq.). A careful check of the fluid balance during the experiment had obviated dehydration as a factor in the fatal outcome (total intake 2,300 cc.; output of urine plus vomitus 1,400 cc.).

Since these experiments were concluded we noticed the very recent publication of Amberson, Nash, Mulder and Binns, who found that the cat, through diuresis induced with "sulfate-Ringer" solution, never gives up more than 25 per cent of its plasma chloride. Judged by the results of our experiments, the cats, if their mechanism of excretion of sulfates is similar to that of dogs, should have had little difficulty in excreting the sulfate at the same rate as it was being administered, thus obviating the necessity of excreting additional chlorides. By the use of total plasmapheresis, however, these investigators were able to reduce plasma chlorides to 7 millimols per liter. Since their artificial serum contained 106.9 millimols of sulfate the assumption seems justifiable that the concentration of sulfate reached in their experiments approached 100 millimols per liter of plasma or 960 mgm. per 100 cc. Some of their animals, at this stage, were still in comparatively good shape. Thus it would seem that in our experiments one reason for the failure to push the concentration of sulfate higher than 350 mgm. per 100 cc. of plasma is related to the failure of the body (kidney) to excrete chlorides as more sulfate is being administered.

One pertinent question regarding the significance of sulfate clearances in dogs should be raised here: viz., to what extent does sulfate clearance define kidney function? A tentative answer may be obtained from a consideration of table 4, in which the results of two different experiments, two weeks apart, on the same dog are recorded. The sulfate clearances at normal concentrations of sulfate in the plasma were practically the same on both days (the average volume of urine per minute was nearly identical). On January 31, 1938, after the administration of an appropriate amount of sodium sulfate, a sulfate clearance of 43.8 cc. per minute was observed at a concentration of sulfates in the plasma of 225 mgm. per 100 cc. and two weeks later at a concentration in the plasma of 28.9 mgm. per 100 cc. the sulfate clearance amounted to 51.0 cc. per minute (all values are averages; the statistical spread is insignificant). Since the sulfate clearances in the control periods were practically the same on both days the conclusion would seem justifiable that at moderately elevated concentra-

TABLE 4

Two experiments on the same dog on different days showing the relative values of sulfate and creatinine clearances

DATE	NUMBER OF PERIODS	PLASMA SULFATE	SULFATE CLEARANCE	CREATININE CLEARANCE	SULFATE CLEARANCE: CREATININE CLEARANCE RATIO
		mgm. per 100 cc.	*cc. per min.*	*cc. per min.*	
1/31/38	4	12.6	6.54	62.6	0.104
	3	225.0	43.8	48.3	0.907
2/14/38	3	12.5	6.8	82.5	0.082
	3	28.9	51.0	76.4	0.668

tions (region of 28.9 mgm. per 100 cc. of plasma) the clearance is 16.5 per cent higher than at much higher concentrations of sulfate in the plasma. Thus, all that one would have gained from these data, statistically significant by themselves, is confusion. However, once the proper emphasis is given to the creatinine clearances in the period under observation it becomes evident that the condition of the kidney in the "control" periods was not the same even though this was not revealed by a difference in "endogenous" sulfate clearance. And, after they have been properly related to the creatinine clearances, the seemingly contradictory sulfate clearances at increased plasma levels can be fitted easily within a comprehensible picture of the excretion of sulfates.

COMMENT. White, in 1923, summarized his studies of the excretion of sulfate in the dog as follows: "The elimination of sulphate in the urine is regulated by factors which are as yet quite obscure." We believe that the present study brings out the importance of a number of definite factors

which are operative in the elimination of sulfates and which in terms of the filtration-reabsorption theory may be described as follows:

Sulfate is present in the glomerular filtrate in the same concentration as in the plasma. Although direct proof is lacking this seems a logical deduction from our knowledge of the distribution of sulfate over the fluids of the body as well as from the ultrafiltration studies reported in this paper and those of Hayman.

Approximately 90 per cent of the endogenous sulfate is reabsorbed by the tubules. Among factors operative in adjusting the exact extent of reabsorption, nonspecific ones might be contrasted to specific ones. The former category, so far as this investigation has been able to uncover, reveals only urinary volume (extent of diuresis) as a definite factor. Further investigation may reveal other important factors. Since reabsorptive processes apparently play so much more predominant a rôle in determining the extent of elimination of endogenous sulfate than the process of filtration, the possibility seems to exist that a better insight into the functional rôle of the tubules might be gained from the study of sulfate excretion in conjunction with the determination of the rate of excretion of substances which measure glomerular filtration.

As far as sulfate as a factor influencing sulfate clearance is concerned (the "specific" factor), the most outstanding feature is the great increase which sulfate clearance undergoes after introduction of extra sulfate into the blood stream. Judged by the general slope of the curve (fig. 3) the ratio of sulfate clearance to creatinine clearance appears to approach unity at increasing concentrations of sulfate in the plasma. Its shape becomes readily understandable if it is assumed that a rather definite amount of sulfate is always being reabsorbed regardless of the absolute concentration in the plasma. If the equivalent of approximately 12 mgm. of SO_4 per 100 cc. is being reabsorbed by the tubules (actually $12/100 \times F$ where F represents creatinine clearance [glomerular filtrate] in cubic centimeter per minute) then at a "normal" concentration of approximately 13 mgm. per 100 cc. of plasma roughly 92 per cent is being reabsorbed. At a plasma level of 24 mgm. of SO_4 per 100 cc. this fraction is reduced to 50 per cent, at 36 mgm. per 100 cc. it amounts to 33.3 per cent, and at concentrations of 240 mgm. SO_4 in the plasma the reabsorption would amount to only 5 per cent of the amount filtered. The ratios of sulfate clearance to creatinine clearance at the respective levels would amount to 0.077, 0.500, 0.667, and 0.950 respectively—figures which fit well into the picture of figure 2.

It would seem that the mechanism of excretion described above simply represents a closer picture of what happens in the excretion of many of the so-called "threshold substances." The threshold, however, is not a rigid one: in the case of sulfate it was found to be lowered after the intravenous

administration of sodium sulfate (i.e., there was less reabsorption for a time), even after enough time had elapsed to allow the concentration in the blood to come back to normal. Somewhat later (twenty-four hours) the threshold had become raised (reabsorption taking place in amounts larger than normal) and finally it returned to normal.

Another striking example of the ease with which "thresholds" adapt themselves to changing circumstances is given by the behavior of the chlorides in the case of the experiment summarized in table 3. During the time that the chlorides in the plasma are lowest—which, incidentally, is the period when the concentration of sulfates is highest—their excretion with the urine is highest and with every step of increase of concentration of chlorides in the plasma (and decrease in concentration of sulfates) a decrease of the amount of chloride excreted is noted. In this connection we might recall the experiment where the failure to get rid of a sufficiently large fraction of the chlorides was considered a prominent factor in the causation of death of the animals receiving large doses of sulfate. Thus, this threshold-mechanism might not at all times be operating to the advantage of the individual.

As far as dietary factors are concerned, our limited data certainly suggest that feeding of extra sulfate with the diet causes a rise in sulfate clearance without affecting the creatinine clearance and without increasing the concentration of sulfate in the blood.

The sulfates present in the urine are derived mainly from the sulfur combined in the proteins of the food and tissues and, under normal metabolic conditions in man and dog, amount to 80 to 90 per cent of the total sulfur excreted. Thus, on a comparable intake of protein different individuals should excrete a comparable amount of sulfate. From the standpoint of renal physiology two ways are open to bring about this elimination: 1, if the level in the blood is relatively low, not too much is reabsorbed by the tubules from the total amount present in the glomerular filtrate; i.e., the clearance is relatively high; 2, if the concentration in the blood is rather high, much reabsorption can take place and still sulfur intake and output may be balanced; i.e., the clearance is low. Interestingly enough, man, with a sulfate concentration in the blood of approximately 3.5 mgm. per 100 cc. and a clearance of approximately 30 per cent of the sucrose clearance (Keith, Power and Peterson), belongs in the former group, whereas the dog, with 13 mgm. of SO_4 per 100 cc. of plasma and a clearance of only 8 to 10 per cent of the creatinine clearance, represents an example of the mechanism discussed under (2). As can be readily seen, the actual amount excreted relative to the extent of glomerular filtration is practically the same in both species. We do not believe that facts are available at present which would explain why species differ in this respect.

As mentioned in the beginning of the article, all results have been ob-

tained with dogs on a uniform diet. It seems probable that other members of this species on similar diets and barring peculiarities of sulfur metabolism will present a similar picture of sulfur excretion. However, under conditions of temporary sulfate (sulfur) retention such as have been reported to occur in pregnancy in the absence of renal insufficiency (3), or in disorders of sulfur metabolism such as cystinuria one might well find a pattern of sulfate excretion essentially different from the one reported here.

SUMMARY

The excretion of sulfate has been studied in dogs. Clearances at the naturally occurring concentrations of sulfate in the plasma amount to approximately 8 per cent of the creatinine clearance. Extent of diuresis (urinary volume per unit of time) has a definite influence on the sulfate clearance.

All, or nearly all, of the sulfate in the serum of dogs can be considered filtrable through collodion membranes.

After the intravenous injection of sulfate its clearance rises markedly and at very high concentrations of sulfate in the plasma it approaches that of creatinine.

The significance of these findings is discussed in terms of the filtration-reabsorption theory and the physiologic implications of this mechanism of a "threshold" are commented on.

The importance of measuring "glomerular filtration" during the periods in which sulfate excretion is studied is stressed.

In view of the great influence of tubular reabsorption in determining the exact extent of the excretion of sulfates at normal concentrations in the plasma—particularly in the dog—it is suggested that more extensive investigation of the elimination of sulfates in connection with studies of renal physiology might prove to be a definite aid in appraising relative tubular activity.

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THE RECTAL TEMPERATURE OF THE NORMAL RABBIT

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The many observations on rabbits have frequently included the seemingly simple measurement of rectal temperature, but the wide range in the values reported leaves doubt as to the normal temperature of this animal. The most detailed studies of the normal temperature of the rabbit are those of Frothingham and Minot (1912), Moore (1918), Bormann, Brunnow, and von Savary (1923), and Lazarus-Barlow (1928). Others who have also made specific contributions to this subject are indicated in the bibliography on page 529. The normal values reported by thirty investigators from 1876 to date range from 36.8° to 41.4°C. Many reports lack descriptions of the methods and the conditions of measurement. As no comprehensive study of the normal temperature or the factors affecting the rabbit's temperature existed, measurements were made during a metabolic study of rabbits at the Nutrition Laboratory to ascertain the factors affecting the rectal temperature, the conditions necessary for comparable measurements, and the average rectal temperature of the normal rabbit.

Animals used. The four breeds of rabbit used—Polish, New Zealand White, Checkered Giant, and Flemish Giant (designated by the letters P, Z, C, and F, respectively, before the animal numbers)—included the smallest and the largest domestic breeds (Castle, 1930). The sex is indicated in table 2, page 528. The thirty-three animals, all adults, ranged in weight from 1.2 to 7.0 kgm., maintained constancy in body weight and physical appearance, and post-mortem examination revealed absence of visible abnormalities. The majority were from 1 to 2 years old, but the ages of three of the Polish rabbits ranged from 3 to 5 years. They were obtained from sources in New England, were kept at the Laboratory, and were amply fed Purina rabbit chow each morning, occasionally supplemented by carrots and carrot tops or parsley. Except for the study of diurnal variations, when only New Zealand Whites were used, the studies of the various factors that might influence the rabbit's body temperature were made on groups composed of three of the four breeds and always included the Polish and the New Zealand White breeds.

Method of measuring rectal temperature. Our rabbits, when held by the front legs, lay quietly on their backs in the lap of an assistant. Frequently they maintained this position unrestrained for several minutes. Gloved hands and the adequately furred back of the animal prevented excessive heat transfer between the assistant and the rabbit. The majority of the measurements were made with a standardized, electric thermo-junction or a multiple thermo-junction, but in some cases a calibrated mercurial thermometer was used. On 23 days the temperatures of five rabbits were measured with both the thermo-junction and the mercurial thermometer, with most satisfactory agreement between the two methods. The thermo-junctions and the mercurial thermometer were lubricated with vaseline or mineral oil to facilitate insertion. To prevent local cooling of the tissues the thermo-junctions, having a small mass, were warmed by gradual insertion, but the mercurial thermometer (laboratory type) was warmed in hot oil to 37°C. before insertion. The thermo-junctions were constructed of copper and constantan wires (no. 24), 0.5 mm. in diameter. By soldering three copper wires, one at one end of the constantan wire, one 75 mm., and the third 125 mm. from this same end, a multiple junction with three thermo-couples at regulated depths was made, employing a single constantan wire. A coating of cellulose cement over the wires made a fairly rigid rod of approximately 5-mm. diameter but with enough flexibility to permit deep insertion safely. At 150 mm. from the end a short piece of thick-walled rubber tubing surrounded the wires, acting as a stop or marker for depth of insertion. Insertion of the multiple junction permitted measurements at three depths (25, 75 and 150 mm.) in the rectum, by use of three switches. Readings were not made until the mercury or galvanometer deflection remained stationary. Occasionally the junction was left in place for two or three minutes longer, but no significant changes occurred. For one series of observations in the study of diurnal variation the single thermo-junction was inserted 25 mm. at a time, and readings were obtained at each depth up to an insertion of 125 mm. and at corresponding depths upon withdrawal of the junction. Readings at the first two depths when the junction was inserted were not used, as the tissues were temporarily cooled by the junction.

FACTORS TO BE CONSIDERED WHEN MEASURING THE RECTAL TEMPERATURE OF THE RABBIT. *Depth of insertion.* The temperature gradient in the rectum of the rabbit has not previously been fully recognized. Establishment of a procedure to obtain representative body temperatures of rabbits and observance of such by all investigators would undoubtedly reduce the wide range of the values recorded in the literature. Forty-nine series of measurements on five of our rabbits, subjected to fasts of varying lengths up to 100 hours and to environmental temperatures

between 20° and 32°C., showed that, irrespective of these different conditions, the temperatures at 75 and 100 mm. in the rectum were for each series essentially the same as those at 125 mm. With the average temperature at the 125-mm. depth in the rectum as a standard, the range in the individual differences and the average difference for the various depths of insertion were as follows:

Depth in rectum...	100 mm.	75 mm.	50 mm.	25 mm.
Range, °C.....	+0.1 to −0.3	±0 to −0.5	±0 to −0.6	−0.2 to −2.0
Average, °C.....	−0.06	−0.16	−0.28	

Representative of the temperatures of seven rabbits living at 28° with food available, measured with the multiple thermo-couples at the three depths in the rectum, are the curves for P-1 in figure 1. The trends of the curves for the 75-mm. depth and for the 150-mm. depth follow

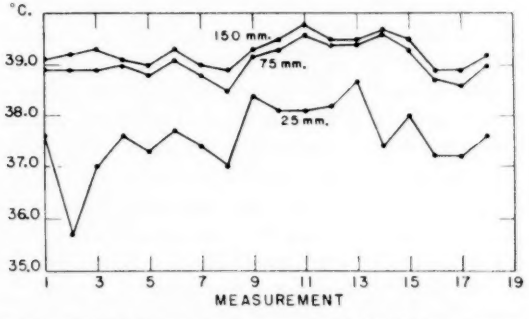

Fig. 1. Comparison of eighteen measurements of the body temperature of rabbit P-1 at three depths in the rectum, on twelve non-consecutive days (by use of the multiple thermo-junction).

each other closely. The average difference between the 75- and the 150-mm. temperatures in 71 measurements on the seven animals was but −0.2°C. Several measurements on six of our rabbits living at 10° showed the same average difference (−0.2°) between the temperatures at 75-mm. and 150-mm. depths as at 28°C. The temperatures at 25 mm. differed from those at 150 mm. more than they had at 28°, the differences being from −1.0° to −4.0°C. At a depth of 25 mm. the temperatures were so variable for all conditions that it is not justifiable to average them as they have little significance, even for comparison with each other. Temperatures measured at 50 mm. in the rectum follow the trends of temperatures at greater depths, although at a lower level. For practical purposes temperatures at 75 mm. or deeper in the rectum can be considered representative of the true body temperature of the rabbit.

Body position. Our data show no difference in temperature due to the position of the animal, whether lying on its abdomen or on its back. The values found are as follows:

RABBIT	RECTAL TEMPERATURE	
	Lying on abdomen	Lying on back
	°C.	°C.
P-1	39.3	39.1
P-3	39.8	39.3
P-5	39.6	39.8
Z-10	40.3	40.2
Z-22	39.9	40.0

To eliminate influence of previous handling, the measurements were made 30 minutes apart. The first three rabbits were measured at a room temperature of 28° and the last two at 17°C. Because position is immaterial and because of the ease and rapidity of making the observations with the rabbit on its back, it is recommended that an assistant hold the rabbit in this position in his lap during temperature measurements.

Activity. After having had their temperatures established when quiescent, several rabbits were chased and agitated, activity being enforced for three minutes. Temperature measurements at depths of 150, 75, and 25 mm. in the rectum were then made for several successive minutes. At the 150-mm. depth the maximum rises above the preactivity levels were from 0.7° to 0.9°C. and in six out of seven instances occurred within 4 minutes after exercise ceased. In the remaining instance the maximum occurred 8 minutes after exercise ceased. Contrary to normal conditions, at some phase of the recovery period the temperatures at the 75-mm. depth equalled and surpassed those at the 150-mm. depth in five instances. The maximum increases in the 75-mm. and the 150-mm. values were within ±0.2° of each other in all but one instance. At the 25-mm. depth the rise was greater but, as noted before, variable and in no case did the temperatures at this depth approach those measured deeper in the rectum. The maximum rise found by us, if only the 150-mm. temperatures are considered, was 0.9°C. The data show that 16 minutes after cessation of activity the temperature was frequently 0.4°C. above the baseline level, and that half an hour is not sufficient to insure return to the initial level.

Diurnal variation. Under comparable conditions of environmental temperature and food, daily measurements on seven of our rabbits averaged only 0.1°C. higher at 4 p.m. than at 9 a.m. Two series of hourly measurements (one at a room temperature of 28° and one at 18°) for 25 consecutive hours with eight animals showed a general increase in body temperature as the measurements were continued. This we ascribe to the repeated stimulus of the hourly measurements, as less frequent measurements did not show this trend. Two series (not consecutive) at a

room temperature of 20°C. were made at 3-hour intervals, one beginning immediately after withdrawal of food and continuing until the 25th hour of fasting and the other representing the 24th to the 60th hour of fasting. The data are plotted in figure 2 with reference to the time of day and the hours of fasting. The curve in figure 2 is based upon the average values at each time of measurement. The body temperatures of these same rabbits were also measured at 6-hour intervals when food was at all times available to them. The maximum limits of these measurements are 39.0° and 40.5°C. The average values are as follows:

Time	11 a.m.	5 p.m.	11 p.m.	5 a.m.	11 a.m.
°C.	39.4	39.4	39.6	39.7	39.6

The data clearly show the absence of any characteristic diurnal variation, whether the rabbit is on food or is fasting up to 60 hours.

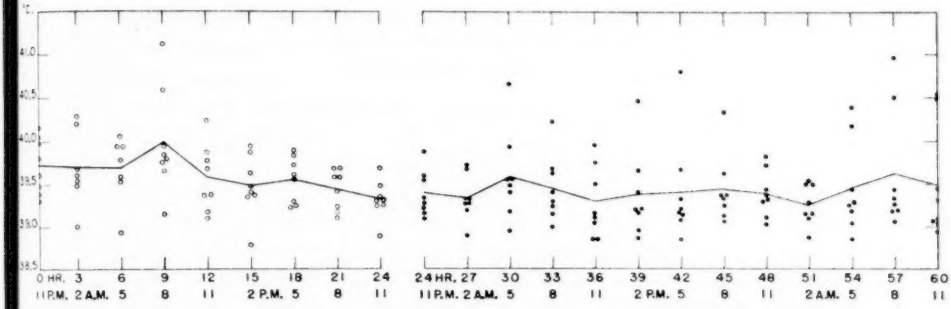

Fig. 2. Influence of time of day and length of fasting upon the rabbit's rectal temperature. The hollow circles represent measurements at 3-hour intervals on eight New Zealand Whites, beginning immediately (0 hr.) after withdrawal of food and continuing through the 24th hour of fasting. The solid dots represent measurements made two days later on the same group of rabbits, beginning 24 hours after withdrawal of food. The curve represents the average trend of the rectal temperatures.

Food. When measured under the same environmental conditions (28°, 20°, 16°, and 10°C.), the rectal temperatures of our animals after 24 hours of fasting averaged 0.26° lower than those when they were on food, the individual variations being from −0.9° to +0.6°C. Since it was not certain that the rabbits had eaten just prior to the measurements on food and just prior to the withdrawal of food for the 24-hour fast, further measurements were made. Three rabbits fasted 65 hours, and their temperatures were measured at the 43rd and 65th hours. They were then given food and were noted to eat. When measured after 2, 4, and 23 hours on food, the temperatures were close to the average fasting value, some slightly above and some slightly below, but in general falling between the fasting values. Although the curve in figure 2 shows a small

decrease in the average rectal temperature during the first 24 hours of fasting, the values when the rabbits were on food (see text table, p. 525) average the same as all the values plotted in figure 2. Higher body temperatures because of the action of fermentation processes (as noted in feces of elephants) were ruled out, as our analyses of respiration chamber air showed that no methane was given off by the rabbit even during the first two hours following the taking of food. Hence the conclusion is that, although food may cause a slight increase in the rabbit's body temperature above the fasting level, the normal variability in its temperature is sufficient to obscure any possible subtle effect of food.

Fasting. That fasts prolonged 60 hours do not lower the body temperature is also shown by the data in figure 2. In fourteen 3-day fasts of rabbits at 24° or 28° this fact was likewise demonstrated, the measure-

TABLE 1

Average rectal temperatures (°C.) of rabbits living at different environmental temperatures for 24 hours or longer

(0 to 72 hours without food)

RABBIT	ENVIRONMENTAL TEMPERATURE, °C.				
	32	28	20	16	10
P-1		39.1			39.2
P-2	39.4	39.3	39.4	39.3	
P-3		39.7			39.4
P-5		39.7			39.6
Z-10		40.4	40.0	40.6	40.0
Z-22		39.9	39.8	40.1	39.7
Z-29	39.8	40.0	39.5		
F-31		39.8		39.2	39.2

ments at the 48th and the 72nd hours of fasting differing from those at the 24th hour by from −0.8° to +0.7° but the average differences being only −0.1° and ±0, respectively. Fasting (at 27° to 30°C.) was prolonged for 25 days with two rabbits and for 19 days with one animal. As the fasts progressed, there were slight decreases in temperature from the 24-hour fasting levels, which amounted to 0.1°, 0.6°, and 0.9°C. in 19 days. On the 25th day the temperatures of the first two rabbits were 0.4° and 0.7° lower than the 24-hour fasting levels. These rabbits maintained a normal level of body temperature during the 3-week fast.

Environmental temperature. Many apparent effects of environmental temperature will disappear when the body temperature is measured at the correct depth in the rectum. Yet environmental temperature may be a significant factor. Summarized in table 1 are the average rectal

temperatures of eight rabbits at various room temperatures. Each average represents at least three and more generally five to ten measurements when the rabbit had been living for at least 24 hours at the same temperature at which it was measured. The averages for P-1, P-3, and P-5 at 28° represent 23, 18, and 29 daily measurements, respectively. The maximum variation between the general averages at the various environments is 0.3°. The individual differences are not significant, as day-to-day variations at the same temperature for the same rabbit may be greater. Eight New Zealand White rabbits, measured frequently at 28° and 18° (after being 24 hours or longer at the given temperature) under conditions otherwise the same, showed no difference in body temperature at the two environments. Hence over the range of 32° to 10° the environmental temperature, provided the rabbit has become adjusted (24 hours or more) to it, does not influence the rectal temperature. Measurements of several of our rabbits during temporary changes in air temperature from 10° to 28° and from 16° to 28° showed no greater variations in rectal temperature than those noted with the same rabbits at a constant air temperature of 28°C. Fifteen measurements of rabbits living at 24° or 28° and placed for 1 to 3 hours at 32° showed temporary elevations in rectal temperature of from 0.9° to 1.7° in nine instances and smaller increases in the remaining six. Rabbits temporarily exposed to 32° can not always adjust their temperature-regulating mechanism immediately, and high rectal temperatures frequently result.

NORMAL LIMITS IN TEMPERATURE AND AVERAGE TEMPERATURE OF THE RABBIT. Summarized in table 2 are the averages of at least five days' measurements, obtained at a depth of 90 to 150 mm. in the rectum, when the rabbits were quiet and adapted to a constant environmental temperature (between 10° and 28°C.). The measurements extended over all seasons of the year. The maximum and the minimum values of 41.1° and 38.4° are well supported by other closely similar values. The range of 2.7°, which may be considered to represent the probable normal temperature range for any *group* of rabbits, is practically only half the range of 4.6° recorded in the literature. The *individual* range for any one of the 33 animals did not exceed 1.8°C. except in two instances, where ranges of 2.6° (P-3) and 2.2° (Z-22) were found. As the measurements on any one rabbit extended for some animals 1½ to 2 years, the range of 1.8° can be considered the maximum normal for most rabbits.

The grand average (weighted) of the daily determinations on the 33 rabbits is 39.6°C. Fifteen other rabbits of various breeds were measured on from 1 to 4 days. The average of their body temperatures under the above prescribed conditions was 39.3° or but slightly lower than the general average noted with the rabbits in table 2.

Sex and size. No sex difference is apparent in our results (table 2). Both the large and the small breeds had temperatures approaching the

TABLE 2

Normal rectal temperature of the rabbit

BREED, RABBIT NUMBER, AND SEX	PERIOD OF STUDY	AVERAGE BODY WEIGHT	DAYS OF MEASUREMENT	RECTAL TEMPERATURE	
				Range	Average
		kgm.		°C.	°C.
P-1♀	5 mos.	1.2	30	38.5–39.7	39.1
P-2♂	17 mos.	1.2	39	38.7–40.1	39.3
P-3♂	4 mos.	1.3	25	38.4–41.0	39.6
P-4♀	17 mos.	1.4	19	38.7–39.9	39.3
P-5♀	25 mos.	1.5	44	38.8–40.2	39.6
Z-6♀	16 days	3.2	9	39.1–40.5	39.7
Z-7♀	16 days	3.2	9	39.0–40.3	39.5
Z-8♀	22 days	3.3	5	39.1–39.9	39.4
Z-9♀	22 days	3.3	6	39.3–40.4	39.8
Z-10♂	6 mos.	3.4	28	39.6–41.1	40.3
Z-11♀	16 days	3.4	9	39.5–41.1	39.9
Z-12♀	11 days	3.5	7	39.2–39.6	39.4
Z-13♀	16 days	3.6	9	39.1–40.6	39.5
Z-14♀	12 days	3.7	6	39.5–40.8	40.3
Z-15♀	19 days	3.7	5	39.7–40.2	39.9
Z-16♀	17 days	3.7	5	39.7–40.7	40.0
Z-17♀	16 days	3.8	9	38.9–39.9	39.3
Z-18♀	16 days	3.8	9	38.8–40.7	39.6
Z-19♀	16 days	3.9	9	38.8–39.4	39.1
Z-20♀	16 days	4.0	9	39.0–40.8	39.5
Z-21♀	19 days	4.0	5	38.6–40.4	39.6
Z-22♂	5 mos.	4.0	21	38.7–40.9	39.9
Z-23♀	19 days	4.1	7	39.4–40.7	40.0
Z-24♀	17 days	4.2	6	39.2–40.1	39.7
Z-25♀	19 days	4.3	6	39.5–40.1	39.8
Z-26♀	22 days	4.4	6	38.9–39.8	39.3
Z-27♀	21 days	4.4	6	39.4–40.9	40.1
Z-28♀	22 days	4.5	5	38.6–40.4	39.6
Z-29♀	13 mos.	4.7	31	38.8–40.5	39.6
C-30♀	1½ mos.	4.6	7	39.8–40.6	40.2
F-31♂	28 days	5.1	16	38.6–40.2	39.5
F-32♂	25 days	5.3	11	39.4–40.3	39.8
F-33♀	2 mos.	7.0	7	39.0–40.4	39.9

extremes, but there was a tendency for the lighter weight rabbits to have a slightly lower average temperature than that of the heavier rabbits.

SUMMARY

To measure the rectal temperature of the rabbit, the animal should be held by the hands of an assistant (preferably in the lap) and not mechanically restrained, the thermometer should either be previously warmed or should be inserted gradually to prevent local cooling of tissue, and, as there is a gradient in the temperature of the rectum, the thermometer should be inserted 75 mm. or more in the rectum.

Activity for three minutes, such as that enforced in this study, produces an average increase of 0.8°C. and renders unreliable as normal the measurements of rectal temperatures for at least 30 to 60 minutes after cessation of such activity.

Ingestion of food causes a slight increase in body temperature, but this is so slight as to be obscured by the normal variability in temperature. A 24-hour fast at 10° to 28°, a 72-hour fast at 24° to 28°, and a 3-week fast at 27° to 30° do not influence the body temperature.

Between 10° and 28° the environmental temperature does not affect the rectal temperature, provided the rabbit has been living at the same environmental temperature for at least 24 hours. But if the air temperature is increased to 32°, a temporary elevation of the body temperature occurs, which disappears upon 24-hour adaptation.

No sex difference and no diurnal variation in rectal temperature, either with rabbits on food or fasting up to 60 hours, have been found.

The limits of normal body temperature of any *group* of rabbits will be from 38.4° to 41.1°C. The range between the minimum and the maximum temperature of the *individual* animal may vary from 0.4° to 2.6°C., but generally is not more than 1.8°C. The average normal temperature is 39.6°C.

Grateful acknowledgment is made to Dr. Francis G. Benedict, former Director of the Nutrition Laboratory, under whose guidance this study was begun.

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HOMEOSTASIS IN THE SYMPATHECTOMIZED DOG

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The importance of the sympathetic division of the autonomic nervous system for maintenance of homeostasis was first pointed out by Cannon (1929). If emergencies do not arise, this division is not necessary and its dispensability in quiet circumstances of life has been emphasized (Cannon, Newton, Bright, Menkin and Moore, 1929; Cannon, 1932). A number of investigations have shown, however, that when sympathectomized cats are subjected to stress none of the adaptations, which occur normally, are completely effective and, in some cases, adjustment fails altogether. Thus, cats lacking the sympathetic system show 1, a lessened ability to withstand high and low external temperatures (Cannon *et al.*, 1929; Sawyer and Schlossberg, 1933); 2, subnormal resistance to anoxemia (Sawyer, Schlossberg and Bright, 1933); 3, a marked increase in sensitivity to injected insulin (Dworkin, 1931; Schlossberg, Sawyer and Bixby, 1933); 4, a lack of compensatory reactions to successive hemorrhages (Schlossberg and Sawyer, 1933; Brooks, 1935), and 5, a diminished capacity for muscular exercise (Hodes, 1938).

On the other hand, studies on sympathectomized dogs have emphasized the normal appearance and behavior of these animals. Brouha, Cannon and Dill (1936) found that the ability of dogs to engage in vigorous muscular exercise is not diminished by sympathectomy. These observers, as well as Bacq, Brouha and Heymans (1934) have also reported that dogs lacking the sympathetic system are not abnormally sensitive to heat or cold.

Because of the apparent difference, after sympathectomy, in the adjustment of the two species to emergencies, it was thought desirable to study more thoroughly the rôle of the sympathetic system in the regulation of homeostasis in the dog. In the present investigation normal and sympathectomized dogs were compared in their endurance of high and low external temperatures, low oxygen pressure, and hypoglycemia resulting from insulin injection. Both sympathetic chains were removed from the stellate to the second sacral ganglia by the technique described by Cannon *et al.* (1929). Unless otherwise noted, all experiments reported were carried out between 2 and 6 weeks after the last operation.

A. The effect of high and low temperatures. That the sympathetic system plays an important part in temperature homeostasis has been established by experiments in which the effects of heat and cold were studied in cats after partial or total removal of the sympathetic outflow (Cannon, Querido, Britton and Bright, 1927; Cannon *et al.*, 1929; Sawyer and Schlossberg, 1933). The effects of changes in external temperature on sympathectomized dogs have not been studied in such detail, but some incidental observations indicate that in them thermal homeostasis is essentially normal. Bacq, Brouha and Heymans (1934) stated that the body temperatures of dogs, so operated, remained normal when the animals were exposed to cold. No information concerning shivering or gross muscular activity was given in the report of these observations. The absence of abnormal rises in the body temperature of sympathectomized dogs during the increased heat production of strenuous muscular exercise (Brouha, Cannon and Dill, 1936) raises doubts concerning the importance of sympathetic function in protecting dogs against hyperthermia. This evidence is particularly strong because the temperature varied within normal limits in these dogs even when they were exercised in a room warmed to 32 to 33°C.

Methods. The responses of normal and sympathectomized dogs, one from each group, were compared during simultaneous exposure. So far as possible the two dogs compared were of approximately the same size and fairly similar as to hair coat.

The effect of cold was observed in a refrigerated room, the temperature of which varied from 3.5 to 6°C. on different days; the variation during any single period of observations was not more than 1.5°. Before each experiment in the cold, pneumographs were fastened about the chests of the two animals and they were tied by short leashes (at ordinary room temperatures of 22 to 25°C.) for ½ hour. At the end of this period of inactivity rectal temperature was taken, to learn the basal body temperature. A centigrade thermometer (graduated in tenths of a degree) was used; it was inserted to a depth of 6 cm. for 3 minutes. The animals were then taken into the cold room and tied in a similar fashion. The exposure lasted 2 or 3 hours, and body temperatures were recorded at 30-minute intervals. Records of respiratory movements were recorded every half hour by means of a tambour attached to the pneumograph. The movements due to shivering were superimposed on the respiratory excursions.

Similar procedures were carried out in the experiments on the effects of heat. One set of observations was made at room temperatures of 40 to 42.5°C.; a second set at temperatures of 37.5 to 39.5°C. In all cases the exposure to heat lasted for 2 hours.

Observations on body temperature were continued for several hours after removal of the animals from either the hot or the cold room.

Results. 1. *The body temperature of sympathectomized dogs.* With the room air between 22 and 25°C. the two groups of dogs studied showed no noteworthy difference in body temperature. In the normal group the readings varied from 38.3 to 39.3°C., with an average value of 38.89° (26 observations on 5 dogs). The average figure for the sympathectomized dogs was 38.85°C., with a minimum of 38.2° and a maximum of 39.4° (26 observations on 8 dogs).

2. *The effect of low external temperatures.* The usual response of normal dogs to cold was a slight temporary rise of rectal temperature succeeded

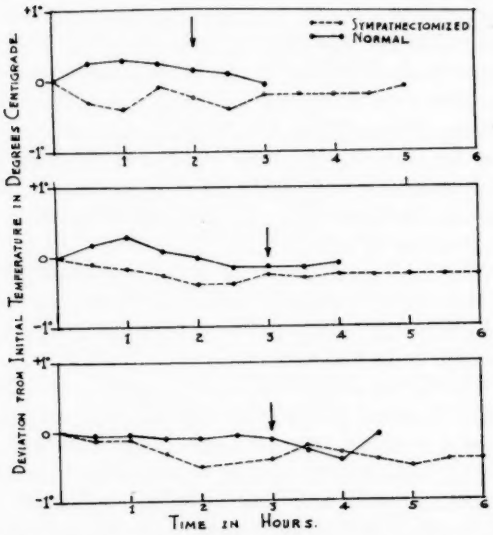

Fig. 1. Comparison of changes in body temperature of normal and sympathectomized dogs in response to cold (4.2 to 5.2°C.). The arrow indicates time of removal from the cold room.

by a fall to the initial level or somewhat lower (table 1 and fig. 1). The change from the basal level was small in all instances; the average maximal rise was 0.24°C. and the greatest fall averaged 0.18°C.

The sympathectomized dogs, studied within 6 weeks of the last operation, typically showed only decreases in body temperature. In one dog (Abel), which had been sympathectomized for more than a year, no decrease occurred; instead, the temperature rose as in the majority of normal dogs, and remained somewhat higher than the initial level throughout the time spent in the cold room (expts. 11 and 12, table 1).

Although, in response to cold, the sympathectomized dogs showed

TABLE 1

Summary of experiments in cold room

EXPERIMENT NUMBER	DOG NUMBER	LENGTH OF HAIR	WEIGHT	DURATION OF EXPT.	INITIAL RECTAL TEMP.	AVERAGE TEMP. OF COLD ROOM	MAX. CHANGE IN BODY TEMP.		SHIVERING		AFTER REMOVAL FROM COLD ROOM		BEHAVIOR OF ANIMAL IN COLD ROOM
							Rise	Fall	Time of onset	Max. rate	Shivering persisted	Time for temp. to return to basal	
Normal dogs													
			kgm.	*hrs.*	*°C.*	*°C.*	*°C.*	*°C.*	*min.*	*per min.*	*hrs.*	*hrs.*	
1	5	Short	7.0	3	38.90	5.2	0.20		2.00	500-600		1½	Fairly active
2	5	Short	7.0	2	38.35	4.7	0.30		2.25	†		1	Quiet
3	7	Short	8.2	2	38.60	4.8	0.20	0.20	4.25	400-500		Fell 0.2°	Quiet
4	7	Short	8.2	3	38.75	4.5	0.20	0.25	3.50	400-500		Fell 0.1°	Quiet
5	8	Long	4.8	2	39.10	4.5	0.05	0.10	28.00	200-300			Restless; temp. normal on removal
6	8	Long	4.8	3	39.10	4.8		0.10	34.00	200-300		½	Quiet
7	9	Long	7.5	2	38.75	4.2	0.10	0.25	19.00	300-400		½	Fairly active
8	9	Long	7.5	3	38.50	5.0	0.35	0.15	23.00	300-400		1½	Quiet
9	10	Long	8.6	2	38.40	4.7	0.60		26.00	200-300		1½	Fairly active
10	10	Long	8.6	3	38.70	4.3	0.40		20.00	200-300		1	Quiet
11	9	Long	7.5	3	38.90	4.6		0.30	14.00	300-400		Fell 0.1°	Quiet
12	9	Long	7.5	2	38.70	5.0	0.05	0.10	16.00	200-300		½	Quiet
Averages							0.24	0.18	16				
Sympathectomized dogs													
1	1	Short	7.3	3	39.10	5.2		0.50	0.75	500-600	3+	Fell 0.1°	Fairly active
2	1	Short	7.3	2	39.15	4.7		0.40	0.50	†	3+	3+	Quiet
3	S1	Medium	8.6	2	39.20	4.8		0.50	1.00	500-600	2	2	Quiet
4	S1	Medium	8.6	3	39.40	4.5		0.60	1.00	500-600	3+	3+	Quiet
5	2	Long	5.0	2	39.40	4.5	0.20	0.45	3.25	700-800	3+	Fell 0.2°	During first hour very active
6	2	Long	5.0	3	39.10	4.8		0.40	2.25	600-700	3+	Fell 0.25°	Quiet except for last ½ hr.
7	4	Long	7.2	2	38.80	4.2		0.50	4.00	500-600	1½	2½	Quiet
8	4	Long	7.2	3	38.50	5.0		0.40	3.50	500-600	1½	2	Quiet
9	S2	Long	9.0	2	38.55	4.7		0.65	3.50	700-800	3½	3½	Quiet
10	S2	Long	9.0	3	38.70	4.3		0.55	2.00	600-700	3	3½	Quiet
11	*	Abel. Long		3	38.35	4.6	0.35		0.50	600-700	½	1	Quiet
12	*	Abel. Long		2	38.40	5.0	0.40		0.25	600-700	¼	1½	Quiet
Averages								0.47	2.17				

* Sympathectomized for over 1 year; figures omitted from averages. Loaned by Dr. L. Brouha. Dogs S1 and S2 sympathectomized by Dr. F. A. Simeone.

† Not recorded.

slightly greater decreases in rectal temperature than the normal controls, the changes were never great in either group. The average maximal fall for the operated animals (the experiments on Abel omitted) was only 0.47°C., in contrast to 0.18° for the normal dogs. The difference in the ability of normal and sympathectomized dogs to maintain a fairly constant temperature in cold surroundings is not impressive.

Both groups of dogs responded to cold by shivering. It began earlier in the sympathectomized animals; the average time for its onset was 2 minutes as compared with 16 minutes for the normal dogs. In addition to the shorter latent period in sympathectomized dogs, the shivering response was more rapid and more energetic in this group (table 1).

Shivering stopped immediately on removal from the cold room in all the normal animals, but persisted for various lengths of time in the sym-

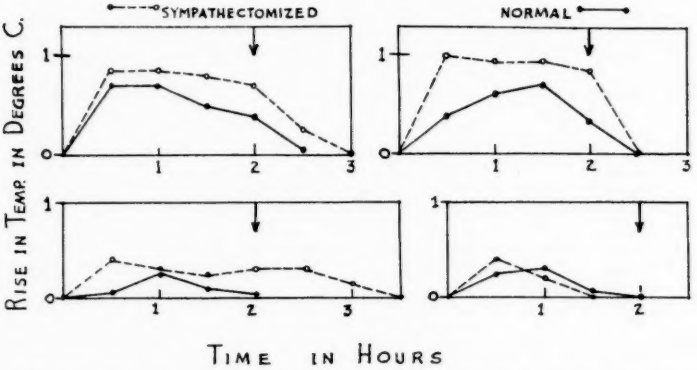

Fig. 2. Comparison of increases of body temperature of normal and sympathectomized dogs in response to heat. Temperatures of hot room; 40 to 42°C. in experiments recorded in upper charts, 37.5 to 39.5°C. in lower charts.

pathectomized dogs. The body temperature also returned to normal more slowly in the latter.

3. *The effect of high external temperatures.* The results of experiments carried out at environmental temperatures higher than 40°C. are summarized in table 2. A rise in body temperature occurred in both the normal and the sympathectomized dogs, the increase varying from 0.7 to 1.6°C. in the former group and from 0.8 to 1.6°C. in the operated animals. Since the temperature increases were within the same range and the average maximal rise was only 0.19°C. greater in the sympathectomized dogs, the degree of hyperthermia in the two groups appears to be essentially similar under the experimental conditions here imposed. The course of the temperature changes in typical experiments is charted in figure 2.

No noteworthy difference between the normal and the operated dogs was observed in the time required for the increased temperatures to return

TABLE 2

Summary of experiments in hot room at temperatures over 40°C.

Exposure of 2 hours

EXPERIMENT NUMBER	DOG NUMBER	LENGTH OF HAIR	WEIGHT	INITIAL RECTAL TEMPERATURE	AVERAGE TEMPERATURE OF HOT ROOM	MAXIMUM RISE IN BODY TEMPERATURE	PANTING		TIME FOR TEMPERATURE TO RETURN TO NORMAL	BEHAVIOR IN HOT ROOM
							Time of onset	Maximum rate		
Normal dogs										
			kgm.	*°C.*	*°C.*	*°C.*	*min.*	*per min.*	*hrs.*	
1	5	Short	7.0	38.35	40.8	0.75	2.25	298	1	
2	5	Short	7.0	38.70	40.3	0.70	1.50	300	½	
3	7	Short	8.0	39.30	42.0	0.80	3.50	368	1	
4	7	Short	8.0	38.60	42.1	1.60	3.25	322	1	Sweating, in estrus
5	8	Long	5.0	39.10	40.6	0.60	1.00	364		Temp. normal on removal
6	8	Long	5.0	39.20	41.7	0.70	0.75	378	¾	
7	9	Long	7.5	39.00	41.3	0.90	1.00	318	1	
8	9	Long	7.5	38.90	40.8	0.75	1.50	312	½	
9	10	Long	8.6	39.20	40.7	1.50	*	306	3+	Sweating
10	10	Long	8.6	38.95	41.5	0.95	0.75	300	1½	
Averages						0.92	1.7	326		
Sympathectomized dogs										
1	1	Short	7.5	38.20	40.8	1.60	5.25	339	1½	
2	1	Short	7.5	38.90	40.3	1.00	4.50	314	1	
3	S1	Medium	8.6	38.80	42.0	1.50	6.00	304	1½	
4	S1	Medium	8.6	38.70	42.1	1.00	2.50	298	1	
5	3	Long	6.4	39.10	40.6	0.90	4.50	332	1	
6	3	Long	6.4	38.80	41.7	0.85	3.50	320	1	
7	4	Long	7.2	39.25	41.3	1.00	3.00	364	1	
8	4	Long	7.2	39.30	40.8	0.80	2.00	340	½	
9	S3	Long	11.4	38.90	40.7	0.90	*	356	3+	
10	S3	Long	11.4	38.70	41.5	1.05	2.25	337	1	
Averages						1.11	3.6	330		

* Panting at ordinary room temperature (27.5°C.); hot, humid day.

to the basal level after removal of the animals from the warm room. In all cases, the length of this time seemed to be more closely related to the degree of temperature rise and to the temperature of the room to which

the animals were removed than to the presence or absence of the sympathetic system.

Sweating (on the foot pads) was observed in only 2 of the normal dogs—in both cases during exposures which caused rises in temperature of 1.5°C. or more (expts. 4 and 9, table 2). This response was always absent in the sympathectomized animals.

The rate of panting was not significantly different in the two groups of animals. The normal dogs began to pant after a somewhat shorter latent period (1.5 minutes on the average) than the sympathectomized animals (average latency of 3.6 minutes). The difference in this time is so small, however, that trustworthy conclusions cannot be drawn from it.

When the experiments in a warm environment were performed at somewhat lower external temperatures (37.5 to 39.5°C.) the results were generally similar to those observed in the warmer surroundings, except that the maximal increases of body temperature were not so great (table 3 and fig. 2).

B. THE EFFECT OF ANOXEMIA. The importance of the sympathetic system in maintaining homeostasis when the oxygen supply is greatly reduced is shown by the much lower resistance to anoxemia in sympathectomized than in normal cats (Sawyer, Schlossberg and Bright, 1933). These investigators found that, in cats lacking the sympathetic chains, fainting occurred after exposures to low oxygen mixtures (6 to 8 per cent) for 14 to 38 minutes, although unoperated animals endured equally low oxygen tensions for at least one hour. In view of the undiminished capacity of dogs to engage in vigorous exercise after sympathectomy (Brouha, Cannon and Dill, 1936) it appears that the sympathetic system is not essential, in this species, for the physiological adjustments which provide a greater delivery of oxygen to contracting muscles. The present study was designed to ascertain the extent to which this division of the nervous system, in the dog, guards against conditions induced by a severe reduction in the oxygen supply when the animal is not active. Normal and sympathectomized dogs were compared.

Method. The animals were exposed to low oxygen percentages at atmospheric pressure in an air-tight metal chamber with a total capacity of 190 liters. Wooden blocks, fitted into two sides of the box, reduced the air space to 90 liters. The removable cover, held in a water seal, had a glass panel which allowed the animals to be observed during the experiment. A perforated pipe, running the length of the box, provided an inlet for the low oxygen mixture. Another pipe, leading from the top, held a rubber stopper through which two short lengths of glass tubing emerged. Through one of these the gas left the chamber; the other served as a connection between tubing from a pneumograph around the animal's chest and similar tubing connected with a recording tambour outside.

TABLE 3

Summary of experiments in hot room at temperatures of 37.5 to 39.5°C.

Exposure of 2 hours

EXPERIMENT NUMBER	DOG NUMBER	LENGTH OF HAIR	WEIGHT	INITIAL RECTAL TEMPERATURE	AVERAGE TEMPERATURE OF HOT ROOM	MAXIMUM RISE IN BODY TEMPERATURE	PANTING		TIME FOR TEMPERATURE TO RETURN TO NORMAL	REMARKS
							Time of onset	Maximum rate		
Normal dogs										
			kgm.	*°C.*	*°C.*	*°C.*	*min.*	*per min.*	*hrs.*	
13	8	Long	4.8	39.00	39.2	0.35	0.75	293		Temp. normal before removal
14	8	Long	4.8	38.90	39.3	0.30	0.50	287		Temp. normal before removal
15	5	Short	7.2	38.30	38.2	0.30	3.30	251	$<\frac{1}{2}$	
16	5	Short	7.2	38.90	38.7	0.40	2.75	260	$\frac{1}{2}$	
17	10	Long	8.5	38.90	38.3	0.25	2.50	232		Temp. normal on removal
18	10	Long	8.5	38.85	37.7	0.35	1.00	222		Temp. normal on removal
19	9	Long	7.3	38.60	38.1	0.40	1.75	243	$\frac{1}{2}$	
20	9	Long	7.3	38.90	38.3	0.30	1.50	237	$<\frac{1}{2}$	
Averages						0.33	1.50	253		
Sympathectomized dogs										
13	2	Long	5.0	39.25	39.2	0.50	5.25	264	$<\frac{1}{2}$	
14	2	Long	5.0	39.30	39.3	0.40	2.00	240		Temp. normal on removal
15	1	Short	7.5	38.70	38.2	0.55	5.00	296	1	
16	1	Short	7.5	38.85	38.7	0.40	4.50	280	$\frac{1}{2}$	
17	*	Abel. Long		38.80	38.3	0.40	6.00	225	$\frac{1}{2}$	
18	*	Abel. Long		39.00	37.7	0.30	7.00	234	$<\frac{1}{2}$	
19	3	Long	6.5	38.50	38.1	0.40	2.50	282	$\frac{1}{2}$	
20	3	Long	6.5	38.95	38.3	0.35	2.00	271	$<\frac{1}{2}$	
Averages						0.41	4.30	261		

* Sympathectomized for over 1 year; loaned by Dr. L. Brouha.

Mixtures of oxygen and nitrogen were made in a gas-mixing apparatus and stored under pressure in cylinders. Thorough mixing was assured by placing the filled cylinders on their sides for a week before samples were

taken for analysis. Oxygen percentages from slightly less than 4 per cent to about 6.5 per cent were used. Ventilation of the chamber was provided by a flow of gas from the cylinder at a rate kept uniform by adjustment of a reducing valve. The outgoing air was collected in a large spirometer in which the speed of accumulation was frequently noted by reading the attached scale. Preliminary experiments, with an animal in the box and compressed air passing through at different rates, established the rate of ventilation necessary to prevent a considerable accumulation of carbon dioxide.

The dogs were trained to sit or lie quietly in the box by several experiences there while ventilation with air of ordinary composition was maintained. For the experiments on exposure to low oxygen a pneumograph was fastened around the dog's chest and the animal was placed inside the chamber. After the tubing was connected with the recording tambour, the lid of the box was sealed. The chamber was ventilated with compressed air for 15 to 30 minutes until the animal was quiet; at this time the respiration was recorded on a kymograph. In order to produce a quick reduction in the oxygen content of the air breathed by the dog, a stream of nitrogen was passed through the chamber for 10 minutes at such a rate that the oxygen was reduced to 5 or 6 per cent by the end of this period. Ventilation with the low oxygen mixture was then begun. Throughout most of the experiment the respiration was recorded every 10 minutes; towards the end of the exposure, records were made more frequently in order to detect the earliest sign of respiratory failure. Just before the animal was removed from the chamber duplicate samples of outgoing air were taken. They were analyzed in a Haldane analyzer.

Results. 1. *Six per cent oxygen.* Four experiments, lasting from 5 to 5½ hours, were carried out with nitrogen-oxygen mixtures containing about 6 per cent oxygen (expts. 4, 5, 6 and 7, table 4). Two normal and two sympathectomized dogs withstood equally well this oxygen tension for that period. A marked increase in the respiratory rate was recorded; no indications of distress were observed, however, and none of the dogs collapsed. On the whole, the animals were calm and relatively inactive; occasionally they moved but in no case did they show excitement or struggle.

2. *Four per cent oxygen.* That extremely low oxygen tensions are endured by dogs has been reported by Greene and Gilbert (1922). In their experiments (under chloretone anesthesia) anoxemia was produced by the rebreathing method; respiratory movements were supported until the oxygen tension was reduced to an average of 3.26 per cent. In the present experiments, therefore, the oxygen percentage was reduced in order to learn whether sympathectomized and normal dogs differed when the test was sufficiently severe to cause collapse. Preliminary experiments on

normal dogs, subjected to an atmosphere containing about 4 per cent oxygen, showed that a critical stage was reached after a few hours. These dogs were observed as long as possible (until ordinary respiration was replaced by gasping), with the purpose of determining a suitable end point. Fainting proved to be not a satisfactory criterion because some animals later became alert while in the chamber and remained so for a considerable time before the final collapse. Moreover, the dogs usually lay so quietly that loss of consciouness could only be shown by a lack of response to stimulation (e.g., the sound made by striking the metal box), and frequent movements seemed inadvisable. Later experiments, therefore, were

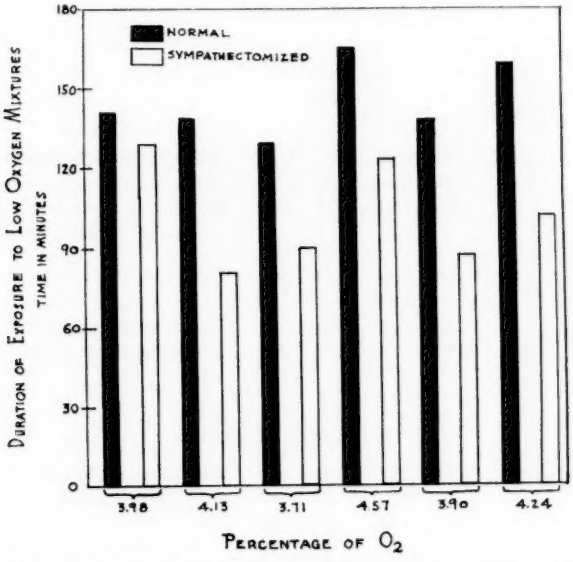

Fig. 3. Comparison of resistance of normal and sympathectomized dogs to low oxygen tensions.

terminated when the respiration began to fail, as shown by sudden and pronounced slowing of rate and marked decrease in depth.

In this series of experiments, each tank of gas mixture served for a pair of observations, one on a normal and the other on a sympathectomized dog. The latter in no case endured the exposure as long as the former (fig. 3 and table 4). The average endurance was 145 minutes for the 6 normal and 102 minutes for the 6 sympathectomized dogs. No noteworthy differences were observed in the respiratory responses of the two groups. An increase in the rate of respiration began during the period of rapid reduction of the oxygen percentage by nitrogen and rose gradually to a

maximum, reached at various times during the second hour after the start. The most rapid rates were never uniformly maintained but alternated with periods of somewhat slower breathing. Maximal increases of 65 to 127 respirations per minute were recorded in the experiments done

TABLE 4

Summary of experiments on the effect of exposure to low oxygen tensions

EXPERIMENT NUMBER	DOG NUMBER	SUPPLIED AIR PER CENT O_2	FINAL AIR		MAXIMUM INCREASE IN RESPIRATION	DURATION OF EXPERIMENT; MINUTES BEFORE RESPIRATION SLOWED	REMARKS
			Per cent O_2	Per cent CO_2			
Normal dogs							
					per min.		
4	8	6.23	5.10	1.07	121		No collapse in 5½ hours
6	5	5.90	4.71	0.94	90		No collapse in 5 hours
10	5	3.98	2.99	1.20	88	141	Symptoms of cortical injury for 5 days following experiment
12	8	4.13	3.11	1.46	127	136	
14	7	3.71	2.77	0.98	119	129	
16	9	4.57	3.59	0.90	69	166	
18	10	3.90	2.95	0.89	165	139	Room temperature 27.5°C.
20	11	4.24	3.03	1.07	148	158	Room temperature 26°C.
Averages					116	145	
Sympathectomized dogs							
5	3	6.45	5.53	0.78	105		No collapse in 5½ hours
7	1	6.04	4.79	1.11	68		No collapse in 5 hours
11	1	3.98	3.29	1.08	70	130	Symptoms of cortical injury for 1 week following experiment
13	2	4.13	3.69	0.70	105	81	
15	3	3.71	3.18	0.87	65	90	
17	4	4.57	3.84	0.76	93	124	
19	A	3.90	3.07	0.92	209	86	Hot, humid day; room temperature 28.3°C.
21	6	4.24	3.66	0.85	159	103	Room temperature 26.5°C.
Averages					109	102	

Experiments done at room temperature of 21 to 24°C. unless otherwise noted.

at the usual room temperatures (21 to 24°C.); in four low-oxygen exposures during hot weather, both groups of animals showed greater rises in rate, probably due to higher temperatures within the chamber.

After restoration to atmospheric air the animals lay motionless for 5

to 20 minutes; at this time the rectal temperature was from 1 to 2°C. lower than normal. They were placed in warm cages and rapidly recovered, only lacking muscular coördination in their first attempts to walk. On the day following the experiment the animals appeared normal, except in two instances when the low oxygen tension was maintained after the respiratory slowing until ordinary breathing was replaced by spasmodic gasping (expts. 10 and 11, table 4). These two dogs showed signs of cortical injury for several days. They were unresponsive when called and ate only when food was placed in their mouths; they remained for long periods in one position; and the usual hopping and placing reactions could not be elicited. This quiet state was interrupted by occasional periods of hyperactivity during which they ran aimlessly about, sometimes biting themselves. The nervous effects of anoxemia were apparently reversible since normal behavior was gradually resumed. In animals subjected to equally low oxygen tensions but restored to room air at the beginning of respiratory failure such symptoms did not occur.

C. THE EFFECT OF INSULIN. Failure of sympathectomized animals to mobilize sugar under conditions which ordinarily cause its liberation from storage has been proved by the absence of hyperglycemia during emotional states (Cannon *et al.*, 1929) and struggle (Bodo and Benaglia, 1938) and by the great sensitivity of such animals to injected insulin (Dworkin, 1931; Schlossberg, Sawyer and Bixby, 1933). These studies were made on cats. The only observations on the blood sugar of sympathectomized dogs concern its concentration during exercise; then the glycemic level varies within normal limits (Brouha, Cannon and Dill, 1936). The response of sympathectomized dogs to insulin hypoglycemia was therefore included in this study.

Method. The effects of insulin on normal and sympathectomized dogs were studied after the animals had fasted for 18 hours. Insulin (0.5 unit per kgm.) was injected subcutaneously into the hind leg. Blood samples of 0.1 cc. were taken from the ear by opening the marginal vein with a sharp blade. All the dogs had undergone previous experiments (in the hot and cold rooms) and were thoroughly accustomed to their surroundings; in no instance did they show emotional disturbances during the simple procedures of removal of blood and injection of insulin. Blood samples were taken before the insulin injection and every half hour thereafter for 5 hours unless convulsions occurred; in that event, the last sample was taken at the start of the seizure. Blood sugar was quantified by the micro-method of Folin and Svedberg (1930). Duplicate samples were assayed in every case.

Results: Blood-sugar values of sympathectomized dogs. The blood-sugar values determined in normal dogs were found to vary from 72 to 98 mgm. per cent, with an average of 85. The sympathectomized dogs had a

range from 73 to 91 mgm. per cent; the average was 83. The similarity of the fasting blood-sugar levels in the two groups agrees with the observations of Cannon *et al.* (1929) and Schlossberg, Sawyer and Bixby (1933),

TABLE 5

Summary of experiments on the effect of insulin

0.5 unit per kgm.

DOG NUMBER	WEIGHT	BLOOD SUGAR			SYMPTOMS	
		Before insulin	Lowest level after insulin	Time taken to reach lowest level	Time of onset	Description
Normal dogs						
	kgm.	*mgm. per cent*	*mgm. per cent*	*hrs. min.*	*hrs. min.*	
5	7.4	98	32	1 30	1 30	Weakness, salivation, unsteady gait
5	7.6	97	41	3 30		None
8	4.8	78	44	3 00		None
8	4.8	82	43	2 00		None
7	8.7	83	41	2 00		None
7	8.4	84	36	3 00	2 30	Weakness, salivation, unsteady gait
9	7.6	82	52	3 30		None
9	7.3	86	42	3 00		None
Averages.....		86	41	2 40		
Sympathectomized dogs						
1	7.3	81	26	1 13	0 55	Marked symptoms, convulsions
2	4.9	91	35	1 20	1 10	Marked symptoms, collapse
2	4.8	87	30	1 10	1 00	Marked symptoms, vomiting; diarrhea, convulsions
3	6.4	85	26	1 17	1 08	Marked symptoms, diarrhea, collapse
4	7.4	73	24	1 33	1 28	Marked symptoms, convulsions
6	6.3	79	28	1 22	1 15	Marked symptoms, convulsions
Averages		83	28	1 19		

Blood-sugar values determined by the method of Folin and Svedberg.

who found no noteworthy difference in the glycemic percentages of normal and sympathectomized cats.

2. *The reaction to insulin.* The effects of insulin injection are sum-

marized in table 5. In 8 experiments on 4 normal dogs slight hypoglycemic reactions occurred in 2 cases—salivation, restlessness, and weakness and incoördination when the animals attempted to walk. These symptoms first appeared from 90 to 150 minutes after the insulin injection and passed off spontaneously. None of the normal animals developed convulsions after a dose of insulin (0.5 unit per kgm.) which produced marked hypoglycemic reactions and convulsions in each of the 5 sympathectomized dogs studied. In the latter group the first symptoms appeared between 55 and 70 minutes after the administration of insulin; they were always more severe than in the normal controls. After a short period of restlessness the sympathectomized dogs manifested pronounced weakness and a disturbance of gait; salivation was profuse; vomiting and diarrhea occurred in 2 animals. These typical hypoglycemic manifestations were quickly followed by complete collapse, and later by severe convulsions except, in 2 instances, when a 10 per cent glucose solution was injected intraperitoneally at the first appearance of muscular twitchings. This restorative measure was used also in the other sympathectomized dogs after the convulsive seizures were in progress, for, in no case, were there signs of spontaneous recovery.

The average maximal fall in the blood sugar was from 85 to 41 mgm. per cent in the normal and from 83 to 28 mgm. per cent in the sympathectomized dogs. At first the rate of fall was similar in the two groups, but the lowest level of blood sugar was always reached earlier in the operated animals (table 5).

D. Discussion. In the regulation of body temperature, sympathectomized dogs exposed to cold and heat do not differ greatly from the normal. In a cold environment the more rapid onset of shivering and its faster rate in the sympathectomized animals indicate a great reliance on this mechanism in compensation for the lack of sympathetic control. This fact and the absence of a rise in body temperature of sympathetically denervated dogs, such as occurs in the majority of normal controls (fig. 1), give evidence that the sympathetic mechanism normally aids in preserving thermal homeostasis in cold environments. That it is not an essential defense against cooling is shown by the slight difference (0.29°C.) in the average maximal fall of body temperature in the two groups. An increased use of the shivering mechanism in response to cold has been reported in cats with the adrenal glands inactivated (Cannon, Querido, Britton and Bright, 1927) and in sympathectomized cats (Cannon *et al.*, 1929; Sawyer and Schlossberg, 1933); these results suggest that sympathetic activity is the first line of defense against cooling. In spite of more vigorous shivering in both cats and dogs lacking the sympathetic system, such cats show a distinctly greater fall of body temperature (average of 1.63°C. in the experiments of Sawyer and Schlossberg) at equally

cold environments than the sympathectomized dogs studied in the present experiments (average fall of 0.47°C.). In explanation of this difference there may be cited the frequency of shivering of normal dogs at low outdoor temperatures which do not disturb the normal cat. And also there is the fact that the dog, as a much more active animal than the cat, has larger and more powerful muscles, and therefore produces more heat when he shivers vigorously. Accordingly, the sympathetic system appears to be less important for the dog than for the cat exposed to cold and shivering seems the more important mechanism.

Sawyer and Schlossberg (1933) found that sympathectomized cats are less able to withstand exposures to a warm environment than are normal controls. As shown above (p. 535), the response of sympathectomized dogs to a similar environment is only slightly different from that of normal controls; the maximal rise in body temperature is not significantly greater nor is there any noteworthy difference in the rate of panting. Again the dog is revealed as an organism remarkably well equipped to withstand unfavorable conditions. Apparently his superior ability to endure warm surroundings is associated with his great activity and the common need to rid his body of excessive heat by panting. The long tongue, the abundant salivary flow, and the frequently used inherent mechanism for regulating body temperature by rapid breathing give the dog a distinct advantage over the cat. These functions are not changed by sympathectomy. In these circumstances the sympathectomized dog is much more efficient than the sympathectomized cat.

In adjustments to reduced oxygen supply, likewise, the sympathetic system seems to play a less important rôle in the dog than in the cat. The hardiness of sympathectomized dogs, exposed to 6 per cent oxygen for 5 to 5½ hours, does not differ from that of normal dogs; when the oxygen percentage is reduced to 4 per cent, a level which supports life for only a few hours, the degree of endurance is almost as great in the sympathectomized as in the normal animals. The inability of sympathectomized cats to withstand an oxygen tension of 6 per cent for more than 38 minutes (Sawyer, Schlossberg and Bright, 1933) reveals at once the difference between the two species. Here again there are non-sympathetic mechanisms, favorable to the dog, that account for his superiority.

A greater maximal capacity for pulmonary ventilation is indicated by his relatively larger lungs, which comprise 1.8 per cent of the total body weight (Gerhartz, 1926) as compared with 0.76 per cent in the cat (weighed in this laboratory). A greater cardiac output is indicated by a larger size of the heart, which represents 0.6 to 1.3 per cent of the body weight (Colin, cited by Richet, 1898), contrasted with the cat's heart, 0.34 per cent (weighed in this laboratory) to 0.48 per cent of the body weight (Richet, 1898). With respect to oxygen transport, a larger blood volume

and higher hemoglobin percentage favor the dog. Measurements by many investigators, listed by Schulz and Krüger (Winterstein, 1925), have disclosed an average blood volume of 8 per cent for dogs and 5.5 per cent for cats. The same authors report hemoglobin percentages (on a human scale) of 94 for dogs and 56 for cats. Such differences appear to account fully for the striking ability of dogs to engage in strenuous, sustained activity, a type of activity which is not characteristic of the cat. The great capacity of sympathectomized dogs to resist anoxemia can be explained by the fact that these highly effective mechanisms are not altered by sympathectomy.

The results of the present investigation indicate that the sympathoadrenal apparatus is equally important in the dog and cat in protecting the organism against the lowered blood sugar due to injected insulin. It is pertinent to observe that under these conditions the typical difference between the cat as a relatively inactive animal and the dog as an animal accustomed to vigorous muscular exercise is not noteworthy; coincidentally the discrepant responses of the sympathectomized dog and cat disappear.

SUMMARY

Sympathectomized and normal dogs were compared in regard to their ability to resist heat and cold, anoxemia and insulin hypoglycemia.

Exposure of normal dogs to cold usually caused a primary rise in body temperature succeeded by a fall below the basal level (fig. 1, table 1). No initial rise and a somewhat greater drop in body temperature occurred when sympathectomized dogs were subjected to cold under the same experimental conditions (fig. 1, table 1). The maximal decreases in temperature did not differ significantly in the two groups (table 1). In cold environments shivering began sooner and continued at a faster rate in dogs lacking the sympathetic chains (table 1).

There were no noteworthy differences between the normal and operated animals in the rise of body temperature or the rate of panting which followed exposure to high environmental temperatures (fig. 2, tables 2 and 3). Sweating of the foot pads, which occurred only occasionally in normal dogs, was absent in sympathetically denervated animals.

Sympathectomized dogs were able to endure an oxygen tension of 6 per cent for 5 hours as well as normal controls (table 4). No obvious symptoms resulted except an increase in the rate of respiration. Exposure to 4 per cent oxygen was somewhat less well withstood by sympathectomized dogs than by normal animals (fig. 3). The average time of respiratory failure in the former group was 102 minutes and, in the unoperated animals, 145 minutes (table 4). No differences were observed in the respiratory responses of the two groups during the exposure (table 4) or in their recovery after these periods of anoxemia.

Dogs without the sympathetic system were much more sensitive to injected insulin than normal controls. This hypersensitivity was shown by a greater decrease in the blood-sugar percentage and a more frequent occurrence and a greater severity of hypoglycemic symptoms in the sympathectomized animals after the administration of standard doses of insulin (table 5). The fasting blood-sugar level in sympathectomized dogs did not differ from normal.

The remarkable ability of sympathectomized dogs, as compared with sympathectomized cats, to endure unfavorable environmental conditions is discussed. It is attributed to the numerous accessory physiological devices, not under sympathetic control, that are possessed by the dog as a running animal: larger lungs and heart per kilogram of body weight, greater blood volume, higher hemoglobin content, abundant salivary flow and a long tongue for heat loss, and a common resort to shivering for heat production. In insulin hypoglycemia these devices are unavailing; then the effects are similar in the two animals.

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THE UTERINE MOTILITY CYCLE IN THE GUINEA PIG

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For many species of animals, a regular uterine motility cycle paralleling the estrous cycle has been reported (see Reynolds, 1937, for a detailed consideration of the literature). Moreover, this myometrial cycle has been clearly shown to be under the control of the ovarian hormones. Estrin normally imparts motility to the uterus (Reynolds, 1932), whereas progesterone inhibits motility (Knaus, 1929; Makepeace, Corner and Allen, 1936). These studies were carried out on the rabbit, which shows major rhythmic contractions during the characteristic persistent estrus, but much less activity during the early phases of pregnancy or pseudopregnancy. When attempts are made to extend the same investigations to other species, certain differences appear that have given rise to much conflicting opinion. Siegmund (1930) and Durrant and Rosenfeld (1931) working with the rat, and Newton (1933) with the guinea pig report a mere increase in the frequency of contraction during diestrus, with little if any change in the amplitude. On the other hand, Keye (1923) and Corner (1923) working with the sow, Blair (1923) with the rat, Hartman (1924) with the opossum, and others, have found a greater frequency of contractions in diestrus together with a marked diminution in the amplitude of recorded contractions. In addition to these cyclic variations of spontaneous motility, the uterus of the rabbit and certain other species displays a virtual quiescence during pregnancy, and, as is well known, does not react to pituitrin (Knaus effect). No such behavior can be shown for the guinea pig's uterus, which responds to pituitrin at all stages of the cycle and during pregnancy.

In view of the general discrepancy, and the widespread use of the guinea pig's uterus as a pharmacological test organ, experiments were begun to study in detail the motility cycle in virgin and pregnant guinea pigs; and, if possible, to determine some of the factors controlling such a cycle. Twenty-seven animals were isolated, and their estrous cycles followed for two or more cycles. They were then divided into four groups as shown in the table.

The animal was killed by a blow, the uterus immediately excised and

suspended in a bath of warm oxygenated Ringer-Locke solution of the following composition:

NaCl	0.90 per cent	$CaCl_2$	0.024 per cent
KCl	0.0420 per cent	$NaHCO_3$	0.050 per cent
$MgCl_2$	0.0005 per cent	Glucose	0.0031 per cent

Spontaneous rhythmic contractions began within ten minutes, and were recorded on smoked paper. In all cases the load and length of the lever were constant. After a good many representative contractions were obtained (1½ to 2 hours), a suprathreshold dose of pituitrin was added and the resulting maximal contraction recorded. In a number of cases, small variations in the salt content of the bath were also made, in view of the sensitiveness of the guinea pig uterus *in vitro* to small changes in its ionic environment, reported by van Dyke and Hastings (1928). As will be shown later, this profoundly influenced the *in vitro* response. Finally, the condition of each ovary was checked by serial sections.

In analyzing the data, the amplitudes of all the spontaneous contractions in the normal preliminary run were measured and averaged. This value, divided by the maximum possible amplitude (obtained with pituitrin), gave the percentage of the maximum represented by the spontaneous contractions. In addition, the average frequency of contractions was determined in each case for the entire run.

From this table it may be seen that, under the conditions of the experiment, spontaneous uterine contractions rapidly diminished in amplitude to about 50 per cent of maximum during the first week post-estrus. Furthermore, the frequency of contractions simultaneously increased, so that the highest frequency was observed in the uterus having the smallest amplitude. By the tenth day spontaneous motility had largely returned to maximum. This roughly coincided with the period of greatest activity of the corpus luteum; as the physiological activity of the corpora diminished, the motility returned to the estrous condition. That this effect was actually due to progesterone is indicated by identical results obtained by the injection of crystalline progesterone prior to the *in vitro* test (see the last four entries in table 1). This treatment was as effective in the intact animal as in the castrate, both of which would normally have shown major contractions. The gravid uterus was likewise observed to be relatively quiescent, the effect being more pronounced than in pseudopregnancy. This might indicate a relatively greater production of the luteal hormone in the pregnant guinea pig than in the pseudopregnant animal.

Visual observation of the contracting uteri seemed to indicate that the phenomenon of increased frequency and decreased amplitude was produced by a differential contractility of the ovarian and vaginal ends

of the uterus. The ovarian end was often observed to contract and relax several times, followed by a contraction of the vaginal end, each being recorded as a separate contraction of small amplitude. There was, however, no definite ratio between the two. Occasionally both contractions

TABLE 1

ANIMAL NUMBER	CONDITION	$\frac{\text{SPONTANEOUS AMPLITUDE}}{\text{MAXIMUM AMPLITUDE}} \times 100$	AVERAGE FREQUENCY PER MINUTE
1	Estrus	93.5	0.15
2	10 days post estrus	94.5	0.12
3	Castrate 5 days	95.0	0.15
4	Castrate 10 days	96.0	0.13
5	First day post estrus	79.5	0.35
6	First day post estrus	77.0	0.39
7	Second day post estrus	40.5	0.42
8	Second day post estrus	57.0	0.40
9	Third day post estrus	34.0	0.30
10	Third day post estrus	47.5	0.35
11	Third day post estrus	51.0	0.37
12	Fourth day post estrus	29.0	0.45
13	Fifth day post estrus	34.0	0.40
14	Sixth day post estrus	71.0	0.30
15	Sixth day post estrus	63.0	0.25
16	Eighth day post estrus	82.0	0.25
17	Tenth day post estrus	90.5	0.12
18	Eleventh day post estrus	90.0	0.15
19	Pregnant 10 days	20.0	0.73
20	Pregnant 10 days	4.0	1.00
21	Pregnant 18 days	3.0	1.50
22	Pregnant 25 days	14.0	0.80
23	Pregnant 25 days	78.0	0.40
24	Castrate 10 days. Injected with 1 mgm. progesterone for 6 days	22.0	0.85
25	Castrate 5 days. Injected 4 days with 1 mgm. progesterone	7.5	0.70
26	Castrate 5 days. Injected 6 days with 1 mgm. progesterone	10.0	0.70
27	Gonads present. Injected 5 days with 2 mgm. progesterone	4.0	1.50

happened to occur together, or nearly so, producing a relatively large excursion of the recording lever. These results are in accord with those of Blair (1923), who explains the activity of the excised rat uterus on a basis of conduction waves passing from ovarian to vaginal ends.

The effects of varying the ionic enviroment of the excised uterus were essentially similar to the results of Van Dyke and Hastings (1928), already cited, with the further observation that a slight increase (3 to 6 mgm. per cent) in $CaCl_2$ completely masked the progesterone induced quiescence of the uteri. Furthermore, the response of the uterus to near-threshold doses of pituitrin (0.001 unit per 100 cc. of bath) was markedly diminished by a small reduction of $CaCl_2$. The magnitude of this response, however, could not be correlated with the estrous stage of the animal from which the uterus was taken.

SUMMARY

1. The uterine motility of the guinea pig, like that of many other species, undergoes a cycle which may be referred to the physiological activity of the corpus luteum.

2. This motility cycle is characterized by decreased amplitude and increased frequency of contractions during the time of activity of the corpus luteum.

3. The immediate cause of this variation appears to be an impairment of conduction of the contraction wave between the ovarian and vaginal ends of the uterus.

4. Variations in the calcium content of the bath markedly affect the motility cycle of the guinea pig uterus, and may mask it completely.

I would like to express my gratitude to Dr. George W. Corner for his suggestions in this work, and to Dr. Erwin Schwenk of the Schering Corporation for the progesterone used.

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THE INADEQUACY OF SUBSTANCES OF THE "GLUCOSE CYCLE" FOR MAINTENANCE OF NORMAL CORTICAL POTENTIALS DURING HYPOGLYCEMIA PRODUCED BY HEPATECTOMY WITH ABDOMINAL EVISCERATION

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Those who have investigated the metabolism of nerve cells have, hitherto, been forced to approach their problem chiefly by way of experiments on isolated tissue. While their work has revealed many interesting chemical reactions which take place in slices or other isolated preparations of brain, one difficulty has always existed. There has been no way of testing the functional condition of such preparations and so of determining what bearing, if any, the reactions under investigation have on functional activity. It is obviously possible that the process of preparation may so disorganize the tissue that the results of subsequent metabolic experiments may yield an altogether distorted picture. Experiments with the whole animal, on the other hand, while free from these objections yield results, the interpretation of which is of necessity extremely complicated. In spite of this, certain salient facts seem to have been firmly established by such observations. A number of workers have investigated the O_2 consumption and CO_2 production of brain tissue in vivo (1) (2) (3) (4) (5). Although in most of these experiments accurate determinations of the rate of cerebral blood flow has been difficult so that a fairly wide margin of error has to be anticipated, they all agree in assigning a surprisingly high value to the rate of oxygen consumption of the brain and in revealing a respiratory quotient close to unity; findings which suggest that the brain possesses a very active carbohydrate metabolism.

The nervous manifestations of hypoglycemia were first noted by Mann and Magath (6) in liverless animals. These workers were also the first to demonstrate the restoration of consciousness to comatose animals by the administration of glucose. Very shortly after this, the symptoms of insulin shock were recognized and treated with glucose. Mann and his associates (7) tested the restorative properties of certain other substances in hypoglycemia and found that the symptoms were relieved by glucose, mannose, maltose and glycogen and to a small extent by dextrin and galactose. No other substances tested were effective.

The hypothesis that the brain possesses no considerable carbohydrate reserves as do other tissues, but is dependent upon the blood sugar for its carbohydrate supply was advanced by Holmes and Holmes (8) and by Holmes and Sherif (9) on the basis of experiments with the whole animal. These workers showed that the maximum amount of lactic acid which appeared on post mortem anaerobic incubation of the brain depended upon the blood sugar level at the time of death. If, on the other hand, the brain was quickly fixed in liquid air, little lactic acid was formed, but carbohydrate could be recovered in amounts which also corresponded to the blood sugar level at the moment at which the animal was killed.

Kerr (10) and Kerr and Ghantus (11) improved the liquid air fixation technique. They demonstrated the presence of creatine phosphate in brain and they claimed that more of the brain carbohydrate was present as glycogen than had been believed by previous workers to be the case. They too showed that the sum of the carbohydrates present corresponded to the blood sugar except at very low levels, when the brain carbohydrate was somewhat the higher.

In the meantime, it has been observed that many substances, including some which in other tissues are known to play an intermediary rôle in carbohydrate metabolism, are actively metabolized by preparations of isolated brain tissue. Among these are lactic acid, Warburg, Posener and Negelein (12); pyruvic, succinic, fumaric, malic and glutamic acids, Quastel and Wheatley (13); fructose, Loebel (14) and α-ketoglutaric acid, Weil-Malherbe (15). All of these substances, as well as glucose, mannose and fructose will support the oxygen consumption of brain slices in vitro. Several workers, Szent Györgyi (16), Krebs and Johnson (17), have presented evidence that the oxidation of carbohydrate in muscle takes place in steps involving some of these compounds. Elliott and Schroeder (18) have evolved a somewhat similar hypothesis of kidney metabolism.

While the theories of various workers differ in certain important respects, they none the less agree in postulating a cycle mechanism of oxidation of carbohydrate in tissues. In general terms, a molecule of hexose is believed to give rise to two molecules of triose. A molecule of triose reacts, according to the theory of Krebs and Johnson (17), with a molecule of oxaloacetic acid, forming citric acid and then, by a series of oxidations and reductions passes by way of α-ketoglutaric, succinic, fumaric and malic acids to regenerate oxaloacetic acid, which reacts again with another molecule of triose. In the course of the cycle the carbon and hydrogen of the first molecule of triose have formed H_2O and CO_2, with the liberation of energy. The available evidence would justify the conclusion that reactions of a similar, if not identical nature occur in the oxidation of carbohydrate in brain tissue.

The development of the technique by which the electroencephalogram may be recorded has opened a way of investigating the effect of possible metabolites on the functional condition of the brain. Hoagland, Rubin and Cameron (19) and Gibbs, Gibbs and Lennox (20) have shown that during insulin hypoglycemia there is a marked change in the character of the cortical potentials and that if the hypoglycemia is prolonged electrical activity is profoundly modified. When the blood sugar is again raised the cortical potentials return toward normal.

The plan of the present work has been to deprive the brain of the living animal of glucose and to observe the effect upon the electroencephalogram. One or more of the substances which are known to be metabolized by brain tissue in vitro have then been injected intravenously, to determine whether or not they, like glucose, would restore the normal cortical potentials.

The brain can be deprived of glucose by the use of insulin or by hepatectomy. If the blood sugar be lowered by insulin in an animal with an intact liver, many substances, including some of those with which we have been concerned, would be converted by that organ into carbohydrate and passed into the blood stream as glucose, thus vitiating the experiment. In an animal prepared for hepatectomy by preliminary partial occlusion of the inferior vena cava, removal not only of the liver but of nearly all the abdominal viscera is compatible with survival for 24 to 36 hours (21). Such a preparation is particularly suited to our purpose, since the formation or release of glucose, not only by the liver but also by the kidneys, alimentary tract, spleen and pancreas is precluded.

Experimental. Dogs, cats and rabbits have been used for the work. The last proved most satisfactory and have been employed for the great majority of the experiments. A preliminary operation[1] was performed, 21 days or more before the main experiment, at which time the vena cava was almost occluded by a silk ligature just proximal to the entrance of the right lumbo-adrenal vein (22). In some cases, approximately $\frac{4}{5}$ of the lumen of both cava and portal were occluded (23). On the day of the experiment, the abdomen was opened under ether anesthesia and the whole of the abdominal viscera, except the suprarenals, bladder and about three inches of the rectum, were removed by the method described by Mann (24). The abdomen was closed in two layers. An opening about 2 cm. in diameter was made in the skull with rongeurs. Suitably insulated silver electrodes were clipped onto the edges of the bone so that they were in contact with the dura mater over the frontal region. The animal was placed on an animal board over a hot water bottle and its limbs lightly secured. The anesthetic was then withdrawn.

The cortical potentials were recorded by means of a capacity-coupled

[1] All operative procedures were performed by one of us. (S. M.)

amplifier and a Grass ink-writing oscillograph. Blood samples for sugar estimations by the Folin (25) micro method were drawn at intervals from the carotid artery. Blood pressures were determined in a number of experiments by means of a wash-out cannula in the carotid artery, and recorded on a smoked kymograph drum. In all experiments one carotid artery was left intact. Injections were made either by means of a hypodermic needle into the ear vein or into the cannulated jugular or femoral. If the substance administered was an acid, it was neutralized with 2 N NaOH and adjusted to a pH of 7.0. The final concentration represented 100 mgm. of the acid ion per cubic centimeter.

In such a preparation the blood sugar falls steadily and in two to four hours usually reaches levels of from 20–30 mgm. per 100 cc. Since no measures were taken to correct for non-carbohydrate reducing substances, these values represent very low blood glucose levels. Although it is often possible to recover animals with intravenous glucose after convulsions have occurred and respirations have ceased, to allow the blood sugar to reach such a low level is dangerous because death may supervene very quickly. We have, therefore, used two types of experiment. In the first, we have allowed extreme hypoglycemia to occur, have administered the test substance and if no recovery occurred, followed it with glucose. A number of animals were lost because, if the test substance gave a negative result, the animal sometimes died before the glucose could be given. In the second type, we have started the administration of the test substance earlier when the blood sugar was between 50–60 mgm. per cent and the cortical potentials were still active. Suitable doses of the material were given at intervals and the effect on the cortical potentials was followed, as was the rate of change of the blood sugar except in cases in which the substance administered was itself a reducing agent. If the cortical potentials were not restored, glucose could generally be given in time to save the animal and restore a rapid rhythm. The final restoration of the rapid rhythm with glucose served as a control and showed that the injected substance was not toxic to the cells and that they must have been capable of normal function, had the test substance been suitable for their metabolic needs. Having established the fact that the cortical potentials in the eviscerated, hypoglycemic animal can be restored by the injection of glucose, certain substances were tested some of which are known to play a part in the oxidation cycle and some which on other grounds promised to be of interest.

Results. The results of this series of experiments have been consistent in all cases with each test substance. Figure 1 presents tracings of the cortical potentials in a typical experiment on an eviscerated hepatectomized rabbit. It will be noted that when the blood sugar is within normal limits the tracing shows fine waves of a few micro-volts' amplitude

and a frequency of about 60 per second, superimposed upon waves of larger potential (30 to 50 micro-volts) and a frequency of 5 to 7 per second (fig. 1b). As the blood sugar falls, waves of greater potential and slower frequency appear, bursts of slow and fast rhythm often alternating (figs. 1b, c and d). In marked hypoglycemia there are commonly waves of relatively high potential occurring once or twice per second; at even lower blood sugar levels all waves may disappear. At this stage the animal often stops breathing. The administration of glucose results in the restoration of the original rhythm after an interval of a few seconds to

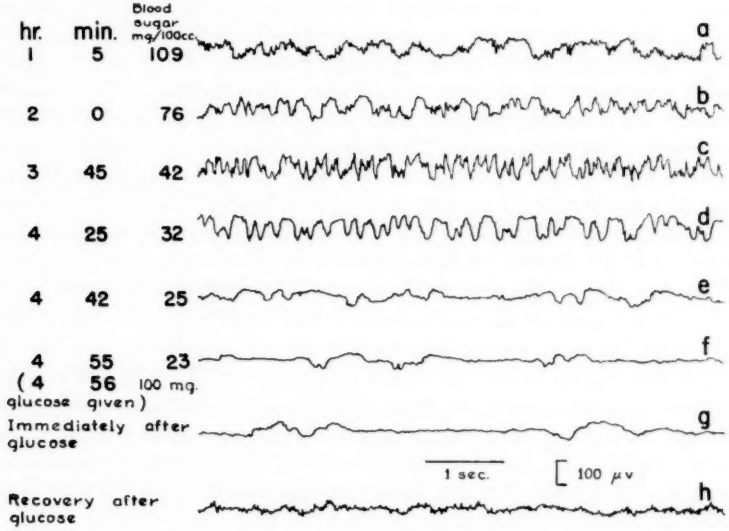

Fig. 1. The oscillograph tracing of an eviscerated, hepatectomized rabbit (wt. 1.1 K). Note slowing of the frequency of the waves with the diminution in blood sugar. In lines *f* and *g* the tracing becomes practically flat. Line *h* (2 min. after intravenous glucose) shows the typical rapid rhythm of the cortical potentials at normal blood sugar levels.

ten or more minutes, depending apparently on the severity and duration of the preceding hypoglycemia and the dose of glucose administered. Even when respiration has ceased and the electroencephalogram tracing is flat, glucose administration often results in recovery if artificial respiration be given for a short time. These results are similar to those published by Hoagland et al. (20) on the effects of insulin hypoglycemia on the electroencephalogram of human subjects, though naturally their experiments were not pushed to the point at which respiration ceased. But the general features—the change from fast waves of low potential to

slow waves of high potential are duplicated, for instance, in experiments on the effects of alcohol and ether by Gibbs, Gibbs and Lennox (19). There is clearly nothing to connect specifically the form of the electroencephalogram which we obtain with hypoglycemia. It is generally believed that the long slow waves of high potential represent the synchronous discharge of large groups of neurones and this seems to be abnormal and to occur in many circumstances in which the cells have to operate under adverse conditions.

It has been shown by Edwards and Page (26) that during insulin hypoglycemia the blood pressure and pulse pressure were lowered owing to an enfeeblement of the powers of the heart, and that the blood pressure rose on the administration of glucose. This raised the question as to whether the apparent effects of hypoglycemia upon the electroencephalogram might really be due to a lowering of blood pressure which was restored by the administration of glucose. Lennox, Gibbs and Gibbs (27) have shown that acute arterial hypotension produces slow waves in man. Beecher, McDonough and Forbes (28) have recently demonstrated the effect of lowered blood pressure on the electroencephalogram in animals. We therefore performed a number of experiments in which the blood pressure was determined by means of a cannula in the carotid. We were able repeatedly to satisfy ourselves that, in hypoglycemia, elevation of the blood pressure, which was usually in the neighborhood of 70–90 mm. of mercury in an eviscerated animal, induced no improvement in the cortical potentials. When the blood pressure was raised by intravenous injections of saline, blood or sodium glutamate there was no improvement, although during hypoglycemia glutamate had a powerful pressor effect. On the other hand, a small dose of glucose often restored a rapid rhythm despite the fact that it might have no appreciable effect on the blood pressure.

Figure 2 shows a tracing obtained with maltose. It will be noted that excellent recovery was also obtained. In tracing *c* the large square-topped waves are due to blocking by the oscillatograph and probably represent rounded waves of even higher potential. Figure 3 shows a typical experiment with one of the non-effective substances (hexose-diphosphate) with subsequent full recovery with glucose.

Table 1 summarizes a typical experiment with each of the substances tested. All compounds listed were used in from 2 to 8 separate experiments. The effective substances were glucose, mannose and maltose. The ineffective ones were galactose, fructose, succinic, fumaric, pyruvic, glutamic acids, hexose diphosphate with and without adenylic acid, glyceric aldehyde, mixtures of glyceric aldehyde and glutamic acid and mixtures of pyruvic and glutamic acids.

Mann and his co-workers (7) found that besides glucose the only sub-

stances which were truly effective in restoring consciousness in the hypoglycemic animal were mannose, maltose and glycogen. We have not

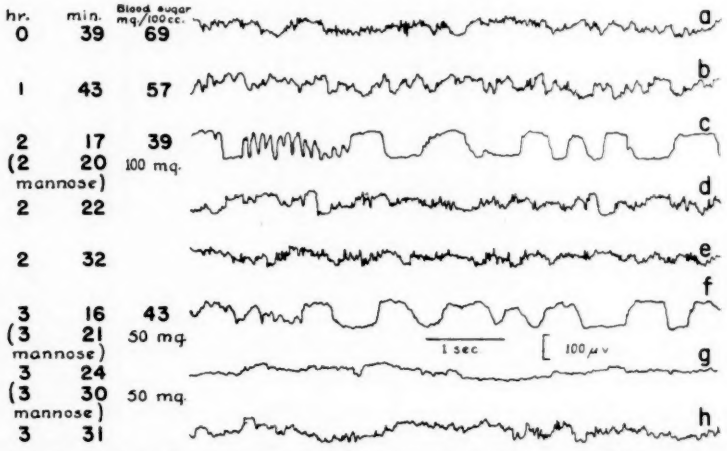

Fig. 2. Tracing of an eviscerated, hepatectomized rabbit (wt. 1.0 K). In line *c* the slow waves of high potential are typical of hypoglycemia. Note return in lines *e* and *h* to fast rhythm of low potential.

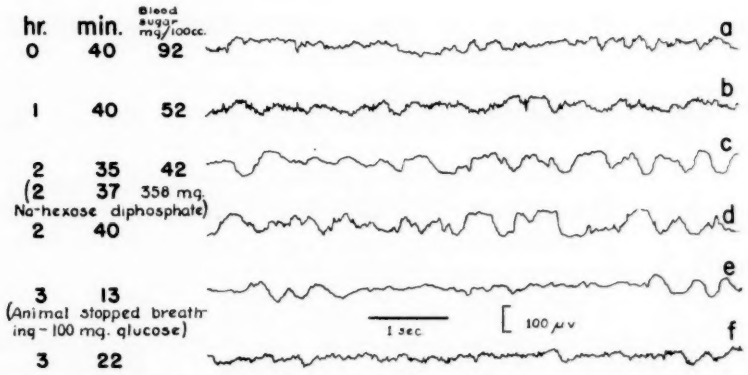

Fig. 3. Tracing of an eviscerated, hepatectomized rabbit (wt. 1.2 K). Note the failure of Na hexose diphosphate to restore normal cortical potentials or to maintain respiration and the return to normal rhythm (line *f*) following glucose. This experiment is typical of all the ineffective substances tested.

been able to test the effect of glycogen in our preparations but we have found that both mannose and maltose are effective. On the other hand, no recovery at all was obtained with fructose. Bollman and Mann (29)

TABLE 1

DATE	SUBSTANCE TESTED	WEIGHT OF EVISCERATED PREPARATION	TIME OF INJECTION POST OPERATIVE	DOSAGE OF SUBSTANCE TESTED	BLOOD SUGAR AT TIME OF INJECTION	RECOVERY OF CORTICAL POTENTIALS	TIME OF GLUCOSE ADMINISTRATION	DOSAGE OF GLUCOSE	RECOVERY OF CORTICAL POTENTIALS AFTER GLUCOSE	REMARKS
		kgm.	*hours minutes*	*mgm.*	*mgm. per cent*		*hours minutes*	*mgm.*		
Aug. 1	Mannose	2.3	2 32	230	49	Good				
			4 10	230	50	Good				
			7 7	230	32	Good				
May 11	Maltose	1.1	3 57	250	35	Good				On each occasion convulsions occurred just before maltose was given. The kidneys were left in situ in this preparation. Similar results with nephrectomy
			6 3	250		Good				
July 22	Fructose	1.0	2 21	100	33	None				
			2 39	100		None				
							2 54	100	Good	
Aug. 9	Galactose	1.4	3 9	200	42	None				Blood sugar at 3 hrs. 29 min. expressed as glucose but represents a mixture of glucose and galactose
			3 28	200	94	None				
							3 30	200	Good	
July 19	Sodium succinate	1.2	4 7	137	34	None				
			4 30	137	32	None				
			4 34		23					
							4 56	50	Poor	
							5 5	50	Good	
	Sodium fumarate	1.2	6 22	138	28	None				Same preparation as used for succinate above
							6 26	100	Good	
July 20	Sodium glutamate	1.1	2 51	130	39	None				
			3 25	130	33	None				
							3 48	100	Good	
			5 5	324	33	None				
							5 25	250	Good	

Aug. 11	Sodium pyruvate	1.3	3 10 3 31	500 1,000	52 52	None None	3 48	500	Good	Animal had already once become hypoglycemic and had been recovered with glucose before pyruvate administered
May 6	Sodium glutamate and pyruvate	1.0	2 10	Glutamate, 860 Pyruvate, 830	48 190	None None	2 50	500	Good	The glutamate-pyruvate mixture run into jugular vein from burette at frequent intervals, 2 hrs. 10 min. to 2 hrs. 44 min. Blood sugar fell progressively; 43 at 2 hrs. 10 min., 36 at 2 hrs. 37 min., and 29 at 2 hrs. 48 min.
Aug. 8	Sodium hexose diphosphate	1.3	4 26	1,156	39	None	4 57	75	Good	At 43 min. blood inorganic P 6.7 mgm. and acid-sol. organic P 31.8 mgm. per 100 cc. At 4 hrs. 40 min. (after Na hexose diphos. injection). Blood inorganic P 5.7 mgm. and acid-sol. organic P 79.4 mgm. per 100 cc.
Aug. 4	Glyceric aldehyde	1.5	3 5 3 12 3 16	400 350	38 104	None None	3 29	400	Good	Blood sugar at 3 hrs. 16 min. due to reducing power of glyceric aldehyde
Aug. 2	Glyceric aldehyde	1.2	1 57 2 5 2 10	60 120 120	32	None None None	2 12 2 19 2 28	100 50 50	Poor Poor Good	
	Mixture of glyceric aldehyde and sodium glutamate		4 6	Glyceric aldehyde, 200 Sodium glutamate, 260	40	None	4 8 4 12	150 150	Poor Good	

state that in their experiments fructose was ineffective in eviscerated animals but was effective in hepatectomized dogs by virtue of the fact that it was converted to glucose in the gastrointestinal tract. This was also confirmed by Drury and Salter (30) in the rabbit. Our failure to demonstrate an effect in the completely eviscerated animal is thus compatible with these findings. In Mann's experiments a transient improvement was noted following the injection of galactose and dextrin. This was attributed to impurities of glucose and maltose. In our experiments a glucose-free galactose was used and no effect noted on the electroencephalogram. No reasonably pure dextrin was available for study.

In the experiments with hexose diphosphate the material used was in the form of the calcium salt. This was converted to the sodium salt by grinding with the theoretical amount of sodium carbonate. The solution was tested for calcium ions, the pH was adjusted to 7.0 and the concentration checked by total phosphorus determinations. Although the values for acid soluble phosphorus in blood indicated that considerable amounts remained in the circulation for some time after injection, as shown in table 1, no recovery of cortical potentials was observed although restoration followed glucose injection as usual. No recovery was observed when adenylic acid in doses of 5 and 10 mgm. was given immediately after the hexose diphosphate.

Table 2 shows the results of some experiments in which sodium malonate was injected. These experiments were performed in order to determine whether malonic acid would exhibit an inhibiting or toxic effect on the intact brain, similar to its action on the brain slice. It is noteworthy that the large doses employed produced no lasting effect. Only when as much as 1.16 grams was injected within 3 minutes into a preparation weighing 1.2 kilos was there the appearance even of a slow rhythm, which disappeared immediately after an injection of 200 mgm. of glucose. This preparation had within the previous 38 minutes received three injections totalling another 1.16 grams of malonate. The concentration of malonate in the preparation must therefore have been considerable (2.3 grams in 1.2 kilo of rabbit), though we have no information as to its distribution through the tissues and body fluids. Thus we have so far, by these methods and the materials at our disposal, been unable to establish the dependence of cortical function upon the oxidation cycle of current theory.

Discussion. In view of the prominent part which some of the substances tested have played in experiments with isolated brain tissue, these results require further comment. Many workers have demonstrated very active succinoxidase and fumarase systems in brain tissue. Succinate and fumarate, however, proved quite ineffective in our experiments.

Quastel and Wheatley (13) have shown that glutamic acid is the only amino acid metabolized by brain tissue and Weil-Malherbe (31) has shown that it is deaminated to α-ketoglutaric acid which substance presumably would then take its place in the cycle. Glutamic acid, however, failed to restore or to maintain cortical potentials.

TABLE 2

DATE	TIME	DOSE OF SODIUM MALONATE	EFFECT ON ELECTROENCEPHALOGRAM	DOSE OF GLUCOSE	EFFECT	REMARKS
	hours minutes	*mgm.*		*mgm.*		
July 27	3 26	285	Rhythm temporarily slowed			
	3 37	356	Rhythm temporarily slowed			
	4 6	356	No further slowing			
July 28	49	342	None			At 1 hr. 30 min. blood sugar was 67 mgm., at 2 hrs. 16 min. 38 mgm. per 100 cc. At 1 hr. 35 min. animal stopped breathing; began again after art. respiration
	1 22	431	None			
	1 35	518	None			
	2 44			380	Recovery	
July 29	4 24	214	None			Animal died at 8 hrs. 26 min.
	4 28	214	None			
	4 34	249	None			
	6 27			200	None	
	6 38	427	None			
	6 49	285	None			
Aug. 2	5 12	285	None			Animal had twice been recovered with glucose before malonate injections were begun. At 6 hrs. 50 min. still alive and breathing well
	5 16	285				
	5 26		Rhythm temporarily slowed	100		
	5 31	570				
	5 50	1,140	Rhythm slowed			
	6 16			200	Recovery	

Braunstein and Kritzmann (32) have described an interesting reaction which takes place in minced voluntary muscle, liver, brain, kidney and heart muscle whereby glutamic and pyruvic acids interact aerobically giving alanine and α-ketoglutaric acids; α-ketoglutaric reacting with a further molecule of pyruvic gives succinic and lactic acids. Mixtures of pyruvic

and glutamic acids, however, again gave negative results in our experiments. Since citric acid is toxic unless administered with calcium and since an upset in the calcium balance might well of itself modify the cortical potentials this substance was not tested. Oxaloacetic acid was not available so that no other members of the cycle were investigated. If, however, the cortical potentials failed because of the lack of material to keep the cycle functioning, addition of any member might be supposed to be sufficient to restore normal conditions. It might be argued that, when the brain is starved of carbohydrate the essential deficiency is of a precursor of the triose postulated by theory, and that lack of the other components of the oxidation cycle is a secondary affair. It might even be that the delay in recovery which is observed after glucose administration in severe hypoglycemia depends upon the delay necessary to form both a sufficient quantity of triose and sufficient of one of the components of the cycle, e.g., succinate—to set the machinery in motion. The term *triose* in this connection is usually assumed to indicate glyceric aldehyde and/or dihydroxyacetone, or their phosphorylated compounds. Of these, only glyceric aldehyde has so far been available. It was tested but was ineffective, as was a mixture of glyceric aldehyde and glutamic acid. The greatly increased reducing power of the blood after the injection showed that the substance circulated for some time and was not all rapidly absorbed by the muscles.

Although phosphorylated trioses were not available, hexose diphosphate was tried. According to Krebs and Johnson (17) the oxygen consumption of their preparations was much increased by the presence of hexose diphosphate or of α-glycerophosphate. Hexose diphosphate was also of obvious interest because of its connection with the metabolism of glycogen in muscle. In the eviscerated rabbit no effect was observable following the injection of hexose diphosphate with or without adenylic acid.

Malonic acid is a powerful inhibiter of the succinic-fumaric enzyme system. Quastel and Wooldridge (33), Quastel and Wheatley (13) and Stare (34) in Szent Györgyi's Laboratory have shown that malonic acid will markedly cut down the oxygen consumption of isolated tissue preparations. Krebs, Salvin and Johnson (35) have injected into intact rabbits 100 cc. of M/5 malonate at the rate of 1 to 2 cc. per minute (i.e., 2.96 grams of malonate in all) over a period of 50 to 100 minutes. They reported no toxic effect and were able to recover succinic, citric and α-ketoglutaric acids from the urine. They state that malonic acid is not metabolized in the body but is excreted unchanged in the urine. The amount of malonate which they gave is considerable, but it was slowly administered and presumably, since it can be excreted by the kidneys did not reach a very high concentration in the tissues at any moment. In our preparations, from which the kidneys were removed, the injection of malonate

should have caused considerable disturbance of the succinic-fumaric system, yet no lasting effect on the cortical potentials was noted.

Loebel (14) showed that fructose was oxidized by brain slices, though it did not give rise to lactic acid anaerobically. The significance of the fact that glucose and mannose both form lactic acid anaerobically, while fructose and galactose do not, is hard to evaluate, since Ashford (36) has shown that lactic acid is formed anaerobically from hexose diphosphate though at a slower rate than from glucose.

It should be made clear that we have placed the electrodes on the cerebral cortex and that our tracings give us no information about the activity of the other parts of the central nervous system. It appears that the medulla, at least, is relatively more resistant to hypoglycemia than is the cortex, since respiration frequently continues after cortical activity has practically disappeared. We have gained the impression, too, that the animal's life (respiration and blood pressure) is prolonged by some substances (e.g., glutamate) which do not prolong cortical activity. We cannot, however, at present evaluate this more precisely.

In view of the experimental evidence which is at present at our disposal, we must conclude that the chemical reactions necessary to permit the normal functioning of the cortical cells do not involve merely the combustion of the known products of carbohydrate breakdown. The work of Mann and his colleagues (7) as well as that of Drury and Salter (30) had already rendered this conclusion probable. It seems likely that some critical reaction occurring earlier in the metabolic chain and probably involving the intact glucose molecule is essential. We might speculate that the reason for this is perhaps that energy can be transferred to the physico-chemical system responsible for the maintenance of function of neurone or synapse only when liberated from some molecule of special form or dimensions, or else that a special product of glucose metabolism is required to form an essential step in the synthesis of some chemical mediator. It has recently been shown (37) that an acetyl choline precursor is formed when brain is incubated aerobically in vitro with glucose or pyruvate but not with other substances. The work of Schweitzer and Wright (38), Miller (39) and Miller, Stavraky and Woonton (40) serve in different ways to connect acetyl choline with spinal and cortical activity respectively.

SUMMARY

1. The electroencephalogram was recorded in rabbits, dogs and cats with progressive hypoglycemia as a result of hepatectomy and abdominal evisceration. The alterations in cortical potentials which occurred in hypoglycemia due to evisceration were similar to those recorded by other workers after insulin and were restored by intravenous injections of glucose.

2. Mannose and maltose had an effect similar to glucose. The following substances were ineffective: fructose, galactose, hexose diphosphate (with or without adenylic acid), glyceric aldehyde, succinate, fumarate, pyruvate, glutamate and mixtures of glyceric aldehyde and glutamate and of glutamate and pyruvate. Others have shown that all these substances are metabolized by isolated brain tissue.

3. Under the conditions of these experiments, considerable doses of sodium malonate injected intravenously had but slight and transitory effect on the form of the electroencephalogram.

4. It was shown that the results were not vitiated by alterations in the blood pressure.

Acknowledgment. It is a pleasure for the writers to express their thanks to Drs. F. H. and E. L. Gibbs for their many helpful suggestions and more particularly for the loan of the cortical potential instrument which made this work possible. We are much indebted to Mr. Albert Grass, the maker of the instrument, for assistance in all technical difficulties. The writers also wish to thank Dr. Hallowell Davis in whose laboratory this work was begun. Special thanks are due to Miss Dorothy Jensen and Dr. Edward Haslam without whose help the work could never have been completed.

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THE RELATIONSHIP OF THE HYPERGLYCEMIC PRINCIPLE IN DIABETIC URINE TO THE PANCREAS AND THE HYPOPHYSIS

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The presence of a blood sugar raising principle in the urine of human diabetics has been reported by Houssay and Biasotti (1933), and by Werch and Altshuler (1937). We have confirmed the findings of Werch and Altshuler in six cases of human diabetes, in all of which an extract of urine made according to the method described below caused a rise in blood sugar on injection into fasting rabbits. Three normal human urines extracted and tested in the same way were without effect.

In view of the fact that anatomical evidence of pancreatic abnormality is usually lacking in human diabetes, the identity of human diabetes with experimental pancreatic diabetes has been questioned. The question is rendered all the more pertinent by the report that pancreatectomy in the monkey does not produce typical diabetes (Collip, Selye and Neufeld, 1937). The question is not clarified by the finding of a blood sugar raising principle in human diabetic urine, since Werch and Altshuler have shown that control of the diabetes with insulin does not cause its disappearance. It seemed worth while, therefore, to repeat the studies of Werch and Altshuler on dogs made diabetic by pancreatectomy, for a more detailed comparison of experimental pancreatic diabetes with the human disease.

METHODS. *Preparation of the urinary hyperglycemic principle.* The procedures outlined by Houssay and Biasotti (1933) were followed with only slight modification. Twenty-four-hour volumes of urine were collected from each dog, and shaken with kaolin in the proportion 5 grams to each 500 cc. urine. The kaolin was centrifuged down, and the adsorbate recovered by solution in 60 per cent ethanol, in the proportion 135 cc. to each 5 grams kaolin. The alcohol was removed by evaporation to a final volume of 25 cc. for each 135 cc. of the alcoholic solution.

Assay. Two cubic centimeters of the extract were injected into the marginal ear vein of rabbits weighing 3.5 to 4.5 kgm., starved for 24 hours. Blood was drawn immediately before injection of the extract, and 15, 30, 60 and 90 minutes thereafter. Total reducing substances were determined

by the micro-method of Folin. In most cases from two to four rabbits were injected with each extract to be tested, the results of the test being recorded as the mean blood sugar response of the group. In a few instances, where rabbits were available whose individual responses to repeated injections of a particular extract had been constant, a single test animal was used.

RESULTS. Urinary extracts from 22 normal dogs have been tested for hyperglycemic action in the rabbit. To establish the upper limits of variation in the rabbit's blood sugar for these control urines, the sign of the predominant response was disregarded, the response being recorded as the maximum elevation of sugar above the control level occurring within ninety minutes after the injection. The mean elevation of blood sugar determined in this way was 4.762 mgm. per cent with a standard deviation of 2.29, and standard error ± 0.489. Taking the mean $+3 \times$ S.D. to mark the upper limit of hyperglycemic response to normal dog's urine, it was assumed that a urine producing elevation of the rabbit's blood sugar by more than 11.63 mgm. constituted a significant variation from the normal. The highest observed response to control urines was +9 mgm. (fig. 1).

Following pancreatectomy, 19 of these 22 dogs consistently produced urines which significantly elevated blood sugar in the rabbit (fig. 1). The urine of only one of the remaining three animals was consistently negative for hyperglycemic action. Pancreatectomy was found at autopsy to have been incomplete in this animal, but appeared to be complete in the other two whose urines were at times positive, at times negative.

The mean rise in blood sugar from all our positive extracts was 37.7 mgm. per cent, with the peak occurring at about one hour, return to normal in about two hours. In degree and time relations the hyperglycemic response was practically identical with the response to 2 cc. of human diabetic urine extract.

Four rabbits received injections at intervals of about three days for one month with extracts of the same depancreatized dog's urine. No decline in the response was observed during this period to suggest the development of refractoriness.

No progressive change in the hyperglycemic potency of the dog's urine following pancreatectomy has been observed. The first urine collected after the operation (within 24 hours) appeared by these crude assay methods to have its hyperglycemic effect for the rabbit fully developed.

The administration of insulin[1] in doses sufficient to keep the urine sugar-free, and of 50 grams per day of fresh raw beef pancreas by mouth for

[1] The authors wish to express their appreciation to Eli Lilly and Company for a generous supply of insulin.

periods of one to two weeks failed to affect the hyperglycemic potency of the depancreatized dog's urine as determined by our present methods.

Pancreatectomy was done in five dogs which had been hypophysectomized from two to four weeks earlier. Three of these animals consistently produced urine which was negative for hyperglycemic action in the

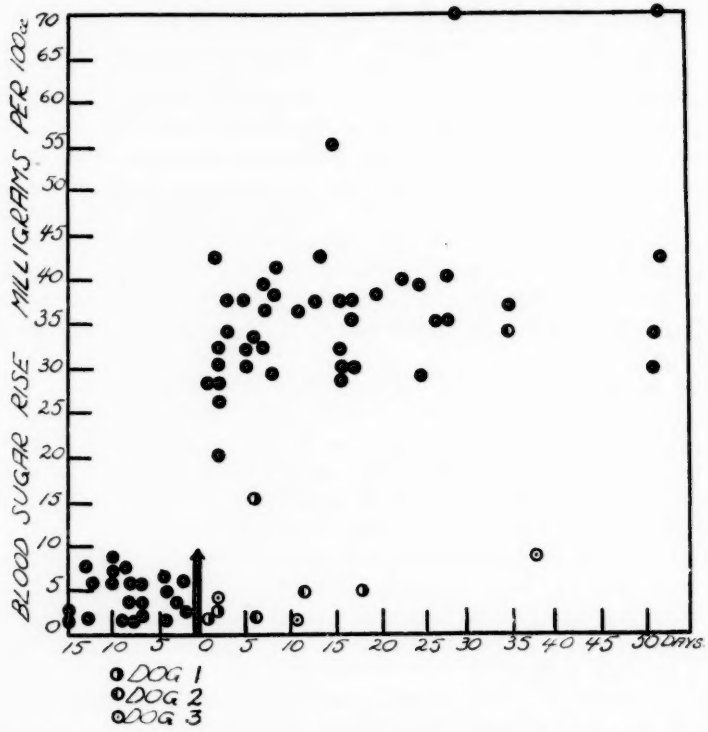

Fig. 1. Hyperglycemic responses in the rabbit to 2 cc. extract of dog's urine, given as milligrams per cent increase in reducing substances. In the control period (before the arrow) only one urine from each dog (total 22 dogs) was collected and assayed. After pancreatectomy (at arrow) from one to seven urines were collected and assayed for each dog. All assays on the three dogs giving conflicting or negative results following pancreatectomy are separately represented in the figure.

rabbit, one produced negative urine throughout except for one day's collection, while the remaining animal produced positive urine consistently (fig. 2). In the absence of complete autopsy data it is not possible to ascribe the single consistently positive urine to incomplete hypophysectomy. Assuming, however, that the results in the group with pancreatec-

tomy alone represent the chance of failure to produce consistently positive urine as 3/22, failure in 4/5 of the hypophysectomized-depancreatized group can hardly be ascribed to chance.

It has been possible to obtain data on only one animal surviving hypophysectomy following previous pancreatectomy. Urine collected after pancreatectomy was found to be positive for hyperglycemia in the rabbit. Following subsequent hypophysectomy all urines collected were negative.

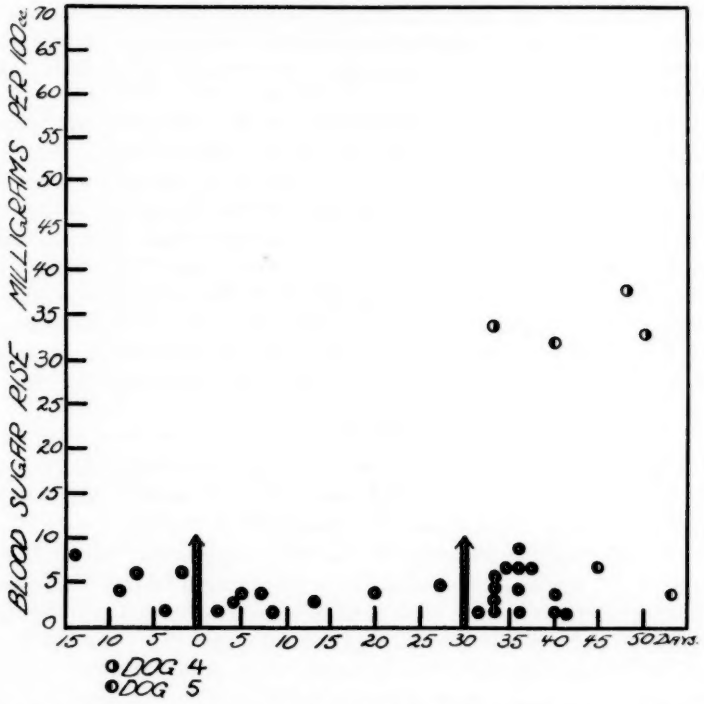

Fig. 2. Construction of figure as in figure 1, summarizing data on five dogs surviving hypophysectomy (first arrow) and pancreatectomy (second arrow). At least one urine from each dog was assayed in each period. The two dogs yielding conflicting results after pancreatectomy are separately represented in the figure.

Discussion. The appearance in dog's urine following pancreatectomy of a hyperglycemic principle for the rabbit, which appears to be identical in solubility, stability, and pharmacological action with the hyperglycemic principle in diabetic human urine is additional evidence for similarity of the experimental disease in the dog and the clinical disease in the human. It is not clear from our data, however, to what function of the pancreas

the urinary principle is related. The failure of insulin to antagonize or reverse this change in the pharmacological properties of urine after pancreatectomy might be regarded as evidence that insulin incompletely reverses the diabetes; or as an alternative, suggest that the urinary principle is unrelated to the incretory functions of the pancreas. The first interpretation is supported by the well known fact that insulin alone fails to maintain depancreatized dogs in good health indefinitely. But the administration of fresh raw beef pancreas by mouth in amounts sufficient, in conjunction with insulin, to maintain depancreatized dogs in good health (Prohaska, Dragstedt and Harms, 1936), failed also to prevent the appearance of the urinary principle. Quantitative data correlating the urinary output of the hyperglycemic principle with the severity of the disturbance in carbohydrate metabolism under various conditions will have to be obtained before complete interpretation is possible.

That the presence of the pituitary is required for the production of the urinary factor in experimental pancreatic diabetes in the dog is shown by the data. Proof that the principle is of pituitary origin, however, awaits identification with one of the hypophyseal factors, upon which work is now in progress. The relative thermostability of the urinary principle, the absence of refractoriness in animals repeatedly injected with it, and the brief duration, with short latency, of the hyperglycemia produced, suggest that it is not identical with the glycotropic factor of the anterior lobe.

SUMMARY

1. The presence in human diabetic urine of a blood sugar-raising principle for the rabbit has been confirmed. Control normal urines do not contain detectable amounts of this principle.

2. Following pancreatectomy in the dog a principle having similar effects on the rabbit's blood sugar may be prepared by identical extraction methods from urine. The principle cannot be detected by these methods in the urine of normal dogs. Insulin and raw pancreas do not cause the disappearance of the principle from pancreatectomized dog's urine.

3. Pancreatectomy fails to cause the appearance of the blood sugar-raising principle in the urine of dogs which have previously been hypophysectomized, while hypophysectomy causes the principle to disappear from the urine of depancreatized dogs.

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ROENTGEN STUDIES OF THE MECHANISM INVOLVED IN SPERM TRANSPORTATION IN THE FEMALE RABBIT

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It has been shown that the passage of sperm into and through the uterus of various animals (Hartman and Ball, 1930; and Rossman, 1937 for the rat; and Florey and Walton, 1932 for the guinea pig; and Evans, 1933, and Whitney, 1927 for the bitch) is due to activities other than their own motility. Conclusive evidence of a similar activity in the rabbit has not been presented. Heape (1898) believed that the sperm were aspirated through the cervix by muscular action. He perhaps did not intend to imply that the uterine activity involved might well carry the sperm to the ovarian end of the uterine horns. In 1905 he stated that they were first found at this point two hours after copulation. Parker (1931) found the same time interval and attributed the rapid transfer from vagina to uterus to an antiperistalsis. Walton (1930) introduced dye into the vagina and did not find it getting into the uterus. He concluded that the spermatozoa penetrate the cervix by means of their own power. Later, Florey and Walton (1932) obtained spermatozoa from uterine fistulae in 20 minutes to 2 hours and regarded these findings as conformation of Walton's original conclusion. In view of the apparent contradictions further investigation appeared justified.

METHODS. Mature virgin rabbits which had been isolated for a period of at least 2 weeks were used. In the first experiments we employed solutions of janus green B or methylene blue. To avoid the necessary laparotomy we turned to the use of iodochlorol and observed its movements, with the aid of the fluoroscope, in the intact animal.

In all cases the dye, or oil, was introduced into the vagina by means of a glass tube. When dyes were used, the rabbit was held during the introduction, whereas with iodochlorol the animal was tied to a board. Visualization through the fluoroscopic screen of the introduction of the first few drops of oil enabled us to determine if the tube had entered the vagina or urethra. The amount of fluid used in any one case varied and is given in table 1.

Usually the introduction of the dye did not stimulate the copulatory reflex. This reaction (erection of vulvar tissue) was elicited only after

artificial stimulation of the vulva with the finger. Thus, all time intervals are dated from the start of stimulation. Observations were continued over a period of an hour.

OBSERVATIONS. The introduction of fluid into the vagina and subsequent artificial stimulation of the vulva induces a reaction in which the contractions of the vagina convey the fluid to the ora of the cervix. These contractions, which are spiral, start at the cervical end of the organ and proceed toward the vulva (fig. 1). They do not pass beyond the point

TABLE 1

RABBIT NUMBER	AMOUNT OF, AND FLUID USED	FIRST APPEARANCE OF FLUID IN UTERUS	UTERUS FILLED IN	VULVAR REACTION
1	2 cc., janus green B		20 minutes*	Yes
2	5 cc., methylene blue		25 minutes*	Yes
3	5 cc., methylene blue		21 minutes*	Yes
4	2 cc., iodochlorol	40 seconds	2 minutes	Yes
5	½ cc., iodochlorol	30 seconds	2 minutes	Yes
5a†	5 cc., iodochlorol	2 minutes	5 minutes	Yes
9	2 cc., iodochlorol	30 seconds	2 minutes	Yes
8	3 cc., iodochlorol	30 seconds	1 minute	Yes
6	4 cc., iodochlorol	No oil seen in uterus		No
7	5 cc., iodochlorol	No oil seen in uterus		No
10	2 cc., iodochlorol	No oil seen in uterus		No
12	Sperm		15 minutes*	Animal bred
15	Sperm		10 minutes*	Animal bred
13	Sperm	No sperm found		Animal bred
14	Sperm	No sperm found		Animal bred
CAT NUMBER				
1	2 cc., methylene blue		10 minutes	Yes

* Indicates time of killing.

† 5a was the same animal as no. 5. Observations of 5a were made 34 days after first positive reaction with iodochloral.

of the vagino-urethral junction, at which point a sphincter completely closes the lumen. This sphincter is seen in all x-ray plates taken and has been noted in the animals opened under local anesthesia. Its closure assures the retention of material introduced into the vagina.

The continuance of a specific contraction wave with a concomitant relaxation of the proximal (cervical) segment of the vagina causes the fluid to flow toward the cervix. As a result an increase in pressure at the ora of the cervix occurs, as seen in figures 2 and 3. These vaginal contractions have been elicited in all animals whether transfer of material into the uterus occurred or not. Those animals in which methylene blue

was used and in which no transfer occurred, the dye stained the ora of the cervix but did not touch the epithelium of the cervical canals. This is

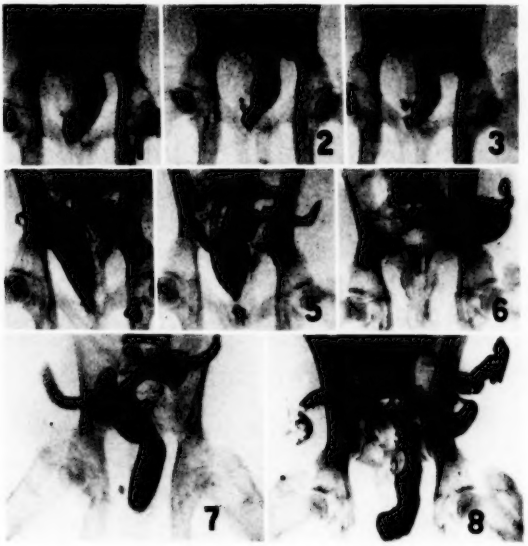

Fig. 1. This, as well as figures 2 and 3, was obtained from an animal in which no oil entered the uterus. The vagina is filled with iodochlorol. The contraction at the cervical end has created a pressure at the vagino-urethral sphincter.

Fig. 2. The contraction wave has progressed caudalward and the oil is beginning to flow toward the cervix.

Fig. 3. The contraction has progressed farther downward and pressure is marked at the ora of the cervix.

Fig. 4. The vagina and uterine horns of rabbit 8 filled with iodochlorol 1 minute after stimulation.

Fig. 5. This figure is from the same animal as figure 4 and was taken 45 seconds later. Note the increase in the diameter of the uterine horns. Most of the right horn lies behind the vagina.

Fig. 6. Obtained from animal 5, 2 minutes after stimulation. Most of the oil is in the left horn. The vagina is almost empty. Note that contractions have severed the connection of the oil in the uterus and the vagina.

Fig. 7. The uterus and vagina of animal 9, six minutes after stimulation.

Fig. 8. This figure was taken 30 minutes after stimulation and obtained from the same animal as figure 6. The interval between the 2 experiments was 34 days. A marked distention of the uterine horns and spiral contractions of the vagina are apparent. The connection between the oil in the uterus and vagina is obliterated by uterine contractions.

a definite indication that the mere increase in pressure at the cervical end of the vagina will not result in passage of fluids into the uterine horns.

The course of events when transfer occurs is unpredictable and rapid. The vaginal contractions elicited by stimulation bring about one or more periods of pressure at the ora. As observed through the fluoroscopic screen, at the height of a specific period of pressure two small blebs appear above the distended cervical end of the vagina. At first the diameter of the blebs, which are in the lumina of the uterine horns, is small and does not increase as the lumen fills. The elongation proceeds at a fairly constant rate, and very soon uterine contractions may be seen traveling toward the cervix; as they do, the upper (tubal) portions of the horns become more and more visible. The upward movement of the oil usually comes to an end within two minutes. Thereafter the transfer of material from the vagina causes an increase in the diameter of the visible uterine lumen.

The extent of the uterine contractions is clearly shown in figure 4 where the beaded appearance of the right horn is apparent. It is to be noted that the upper extremity of the horn contains more oil than the area of contractions. Also, the vaginal contraction which has just started is seen. In figure 5, obtained 45 seconds after figure 4, the left horn contains much more dye than before. In a similar manner, the right horn has increased in length although the increase is not as great as portrayed, because in figure 4 the greater part of the horn lies behind the vagina. The increase in size of the lumen of both horns has begun.

The increase in the amount of the oil in the uterus indicates that the vagina continues to force fluid into the opened cervix. This process, however, does not continue over an extended period. Intervals occur in which uterine contractions sever the connection between the visible vagina and uterus. Thus, periodically additional fluid is carried into the uterine horns. Note the difference in the size of the uterus in figure 4 taken at 1 minute and figure 8 taken at 30 minutes. Although these plates were obtained from different animals the contrast in a single animal is quite as marked.

The amount of fluid introduced into the vagina plays a part in later stages. In figure 6 taken at 2 minutes the greater part of the oil has been transferred into one horn. The vagina is seen only as a thin broken line with the small amount of iodochlorol present confined mainly to the cervical end. The visible uterus in this specimen did not increase in size as the period of activity continued. It is in sharp contrast to that shown in figure 8 obtained from the same animal at a later date, when sufficient oil was introduced to fill the upper reaches of the vagina.

Despite the continued activity on the part of the vagina and uterus over a period of an hour, we have been unable to see any definite indication of the fluid in the tubes. The sphincter at this point remained closed.

Observations made on a single cat showed uterine contractions similar

to those observed in the rabbit. The uterine horns were filled with the dye (methylene blue) ten minutes after the start of stimulation.

DISCUSSION. The response of the reproductive system of an oestrous rabbit to sexual stimulation is of such a nature that if certain fluids are in the vagina when the stimulation occurs they are transported to the ovarian end of the uterine horns in a relatively short period of time. This transportation requires from 2 to 5 minutes and is brought about by the action of the vaginal and uterine musculature. Three stages are present in this activity: 1, the placing of the fluid into the cervical end of the vagina; 2, the transfer from vagina to uterus; and 3, the ascent of the fluid through the uterine horns.

It is essential that fluids are placed above the vaginal sphincter. The closure of this sphincter creates a closed tube and subsequent contractions of the vagina cause a churning of the contained liquid. As a result the fluid is forced to the ora of the cervix and is under pressure at this point periodically. Due to the difference in the combined length of the urogenital sinus and vagina, and the length of the penis, the same factors in all probability are active in the transportation of spermatozoa. There is, thus, an assurance of sperm reaching the cervix whenever seminal fluid is introduced into the vagina.

In the second step, the entrance of the fluid into the cervical canals, vaginal and uterine activity are required. Walton (1930) has shown that excessive pressures in the vagina will not force fluids into the uterus. Our findings substantiate this and show that an effective sphincter is present at the ora of the rabbit cervix. Although we have no evidence of the cervical action prior to the appearance of the fluid in the uterus, it appears that uterine contractions are continued into the cervix and create alternate periods of contraction and relaxation there. In the relaxation of the cervical musculature a negative pressure would be created in the cervical canals and this in conjunction with the excessive pressure in the vagina would cause the fluid to enter the former. Thus, the transfer from vagina to uterus is not a simple aspiration, but rather an equalization of pressures in two continuous tubes as suggested by Florey and Walton (1932) for the rat and guinea pig, and by Evans (1933) for the bitch.

The dipping action of the cervix as described by Heape (1898) may occur. On the other hand, it appears unnecessary for the cervix to change its position to contact the vaginal fluid. As little as 0.5 cc. of iodochlorol was periodically confined to the cervical end of the vagina. Furthermore we have not observed antiperistalsis in this connection and see no reason for a postulate such as given by Parker (1931).

The factors which determine the coördinate activity of vagina and

cervix have not been found. With the possible exception of the vulvar reaction, we know of no way in which to predetermine if the transfer of fluid into the uterus will occur in a given rabbit.

The ascent of fluids through the uterus does not differ from that in other forms, although there is no distention of the lumen. Florey and Walton (1932) have shown that transfer does occur in the rat without distention and it is known to occur in the dog (Evans, 1933). The pressure differentials described for the rat (Rossman, 1937) are active in the rabbit during normal uterine peristalsis although the speed of transportation is not as rapid. The activity is a continuous process and distention of the lumen occurs in time if sufficient material is introduced into the vagina. The vaginal pressure may force fluid a short distance through the uterus but does not account for its reaching the tubal end of the horns.

We have no explanation of why the oil or dye does not enter the fallopian tubes. Bischoff (1845) maintained that in the dog it required hours for the sperm to enter the oviducts, although later observations by Whitney (1927) and Evans (1933) tend to contradict this. In our experiments, during the one hour period of observation, pressures were created at the junctions of the uterus and tubes which would be sufficient to force the liquid into the tubes if a sphincter were not present. Why the sphincter should remain closed for this period of time cannot be answered, although its action may help to explain Heape's (1905) and Parker's (1931) observation of sperm at the tubal end of the uterine horns after two hours. This sphincter invites further investigation.

The observed transfer of fluids into and through the uterus may be interpreted as being similar to the transfer of spermatozoa. We have found spermatozoa at the utero-tubal junction within 10 minutes. We must conclude that our failures, in some cases, as well as those of other investigators, to find sperm are not due necessarily to their absence. The single observation of transfer of dye in the cat indicates that spermatozoa are passive in their movement to the utero-tubal junction in animals which have an extended heat period.

SUMMARY

Transfer of methylene blue, janus green B and iodochlorol through the reproductive system of oestrous rabbits have been observed after artificial stimulation of the vulva. These dyes reach the tubal end of the uterine horns 2 to 5 minutes after the start of stimulation. Combined activity of the vagina and uterus are responsible for this transportation, and indicate that the mechanism of sperm transportation does not differ in all probability from that of other forms.

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CEREBRAL METABOLISM AND ELECTRICAL ACTIVITY DURING INSULIN HYPOGLYCEMIA IN MAN[1]

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Both metabolic and electroencephalographic observations have been made on the brain of schizophrenic patients receiving Sakel's insulin therapy. A marked decrease in the cerebral oxygen consumption has been demonstrated during insulin coma (1, 2, 3). Electroencephalographic studies made by Hoagland, Rubin and Cameron (4) have led them also to conclude from a variety of evidence, including hypoglycemia, that diminished frequency of the alpha waves indicated a decreased cerebral metabolic rate. In the present communication both electrical and metabolic observations were made simultaneously in an effort to determine the degree of correlation between the results obtained by these two methods of study.

The observations presented are those on chronic schizophrenics who were given the insulin treatment at the Harlem Valley State Hospital. The management is essentially the same as that instituted by Sakel, the chief difference being the duration of the coma, for it is found in the cases of chronic schizophrenics that, in order to obtain the best results, a much longer period of coma is necessary than in the treatment in acute cases of dementia praecox (5).

METHOD. Patients who had attained their coma-producing doses of insulin were studied. In most cases before the injection of insulin, electroencephalograms were taken and blood samples drawn. The method of recording brain potentials was the same as that used by Hoagland with an input lead connected to the scalp over the occiput and the indifferent electrodes firmly placed behind each ear on the mastoid process. Electro-

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[2] This work was done at the Harlem Valley State Hospital, Wingdale, New York and we are grateful for the coöperation of Dr. John R. Ross who made possible this investigation, and to Miss Alberta Schultz for the blood sugar determinations.

encephalograms were recorded on paper tape by means of an amplifier and an ink-writing undulator. Since a one-channel system was used, the recordings from the occipital and frontal regions were not simultaneous. Blood samples were collected from the internal jugular vein and the femoral artery, the method of obtaining jugular blood being that introduced by Myerson, Halloran and Hirsch (6). Analyses were made of the sugar (7) and oxygen contents (8) of the blood.

After the initial blood samples were collected and the electrical observations made, the patient was given insulin and each ½ hour thereafter further electrical recordings were made. Blood was drawn for analysis whenever marked electrical changes were observed. These were most apparent when the patient lapsed into coma, some time later just before the termination of coma and again after carbohydrate administration.

Results. In all, 10 experiments were made of 5 patients. The data of only 3 experiments, one on each of 3 patients, will be presented in detail. The excellent agreement among the results obviates the necessity of recording all data. Table 1 discloses the sugar values and cerebral arterial-venous oxygen differences as well as the averages of all observations made throughout the course of the experiments. Figures 1, 2, and 3 contain the results on E. B. September 12, N. W. September 7, and M. W. September 13 before, during and after coma. The oxygen consumption and the alpha frequency vary together for they decrease with the injection of insulin and become greater as a result of the administration of carbohydrate. The delta index,[3] on the other hand, varies inversely to the other 2 variables.

Discussion. That the brain fulfills its energetic requirements largely from the combustion of carbohydrate has been repeatedly emphasized (9, 10, 11). A decreased cerebral oxygen uptake with low levels of blood sugar has been observed in dog and man (2, 3). Furthermore the administration of carbohydrate during hypoglycemia raises the cerebral oxygen utilization in dogs (2), and as disclosed by the present observations in man as well. A correlation between oxygen consumption and electrical changes was attempted because Hoagland and his co-workers have shown that the frequency of the alpha rhythm, other things being equal, is proportional to the rate of respiration of the cortical cells. These conclusions are based on the following observations: *a*, the temperature characteristics for alpha frequencies are identical with the principal values obtained for cell respiration of numerous tissues *in vitro* (12). *b*. The alpha frequencies during the course of insulin shock treatments in man parallel with a lag the falling blood sugar curves. Injected sugar restores the frequencies to normal

[3] For definition and discussion of delta index see Hoagland, Rubin, and Cameron (4).

TABLE 1

The cerebral arterial-venous oxygen differences, volumes per cent and blood sugar, milligrams per cent

A = Arterial, B = Internal jugular vein

NAME	DATE	BEFORE INSULIN	DISAPPEARANCE OF ALPHA WAVES	BEFORE GLUCOSE	AFTER GLUCOSE
E. B.	August 31			*3.76* A—23 B—22	*6.38* A—47 B—44
	September 8			*2.89* A—25 B—82	*4.64* B—52
	September 12	*9.00* A—88 B—81	*2.76* B—22	*1.60* A—28 B—23	*4.95* *6.30* B—45 B—57
	September 16			*2.37* A—38 B—28	*6.04* B—56
M. W.	September 1	*5.26* A—114 B—101	*1.94* A—20 B—18	*1.55* A—26 B—23	*5.77* A—222 B—196
	September 13	*7.42* B—75	*3.30* *7.09* A—34 A—29 B—25 B—26	*1.69* B—26	*6.26* B—37
	September 15			*1.30* A—26 B—22	*4.96* *1.14* B—29 B—20
A. C.	September 2	*7.95* B—40	*2.92* A—23 B—15	A—18 B—17	*4.55* A—72 B—59
M. S.	September 6	*6.99* A—98 B—89	*4.40* A—28 B—25	*1.32* A—29 B—25	*4.00* *6.29* A—89 B—70 B—111
N. W.	September 7	*4.26* A—75	*0.09* A—28 B—26	*0.23* A—26 B—22	*0.01* *3.14* A—50 B—23 B—92
Averages.....		*6.81*	*2.57*	*1.77*	*5.13*

Oxygen utilization in italics; Blood Sugar in roman type.

values (4). *c.* Thyroxin accelerates the alpha frequency along with the basal metabolic rate (13). *d.* Dinitrophenol, a well-known metabolic stimulant, also accelerates the alpha frequencies along a smooth curve (14). The present studies afford direct evidence for Hoagland's views in so far as insulin hypoglycemia is concerned.

Patient E. B. (fig. 1) started with a pre-insulin alpha frequency of 9.5 per second and an oxygen utilization of 9 volumes per cent. Two hours later at the time of complete disappearance of the alpha waves the oxygen

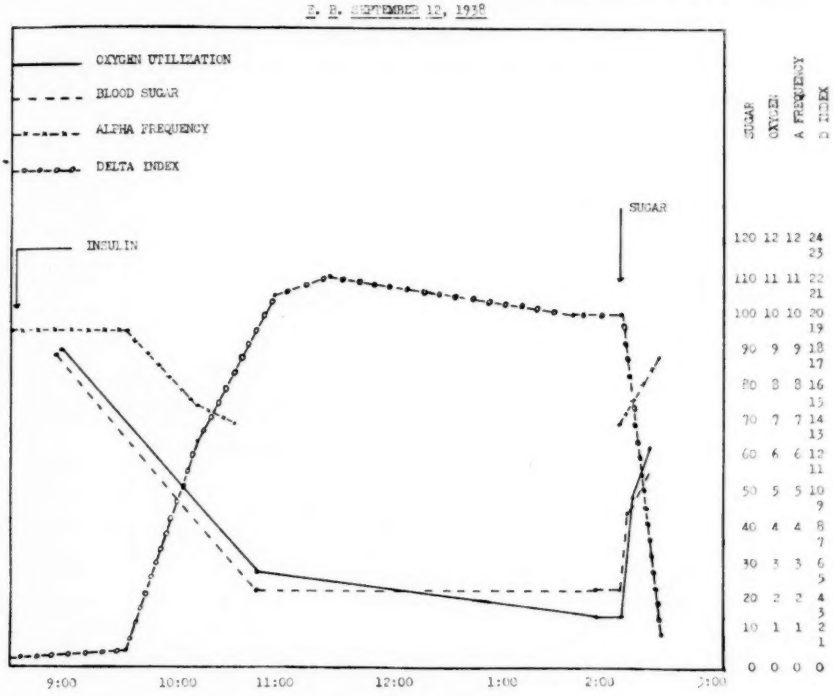

Fig. 1

uptake had diminished to 2.76 volumes per cent. From the pre-insulin period to the time of the complete disapperance of the alpha waves their frequency gradually diminished. Probably a similar decrease of oxygen utilization could be demonstrated if blood samples were obtained more frequently. When all the observations are considered (table 1), the average oxygen utilization before insulin, 6.81 volumes per cent, is close to the value previously obtained on a larger number of patients (3). At the time the alpha waves were no longer countable, the average uptake fell to 2.57

volumes per cent. Similarly an increase of alpha frequency after the administration of sugar is accompanied by a rise of oxygen consumption from 1.77 volumes per cent to 5.13 volumes per cent. E. B. (fig. 1) required a period of 5 minutes after the administration of sugar by nasal catheter before the alpha frequency reappeared at the rate of 7 per second. At that time the oxygen consumption was raised to 4.95 volumes per cent. Thirteen minutes after the administration of carbohydrate both alpha frequency and oxygen consumption were further increased to 8.8 per second and 6.3 volumes per cent respectively.

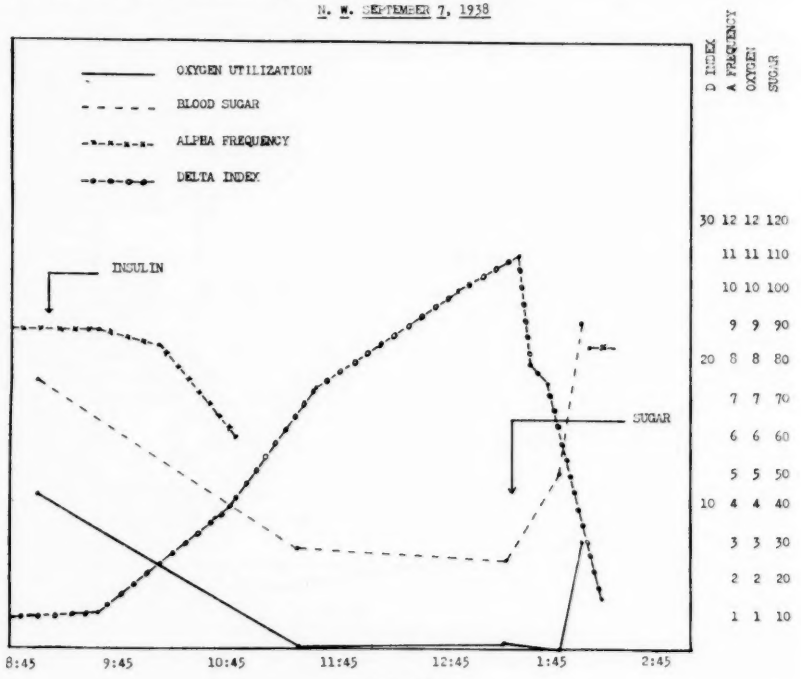

Fig. 2

Whatever the time lag in the reappearance of the alpha waves, a similar delay was observed in the restoration of the oxygen consumption. Twenty minutes after carbohydrate was given to N. W. (fig. 2) September 7 still showed no return of alpha waves nor oxygen uptake. At 40 minutes, however, both had increased towards normal. Another striking correlation is seen in the observation of M. W. (fig. 3) September 13, who after a loss of alpha waves, exhibited spontaneous ephemeral recovery without administration of carbohydrate. Oxygen utilization simultaneously

increased to pre-insulin values. That the oxygen uptake is a better indicator of cerebral metabolism than blood sugar is shown by the fact that the latter remained practically unchanged throughout this episode. Perhaps brain glycogen was broken down to furnish cerebral energy. If sugar was liberated by the liver to cause the increase of cerebral metabolism then this sugar must have been absorbed by the tissues of the body as rapidly as it was liberated.

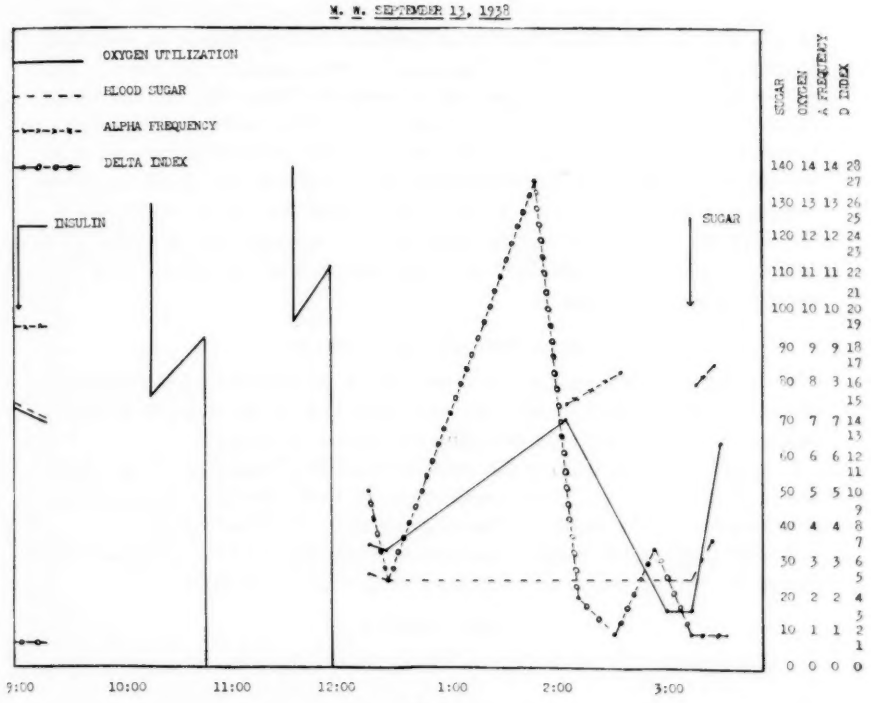

Fig. 3

Hoagland, Rubin and Cameron (11) have demonstrated that the delta index increases at the time the alpha waves disappear. The present observations disclose that the high delta index is accompanied by a diminished oxygen consumption, which continues to decrease from an average value of 2.57 volumes per cent to 1.77 volumes per cent (table 1). The relationship to the other variables here studied is well brought out in the observation of M. W. (fig. 3) September 13. An increase of the delta to 25 and disappearance of the alpha waves is followed by a short spontaneous re-

covery of the alpha frequency (7.5 to 8.2 per second) and utilization of oxygen (7.1 volumes per cent) while the delta index decreases from 25 to 4 and 2. The delta index, however, begins to rise once more to 7 as the alpha waves disappear. Finally when the alpha frequency approaches normal values following the sugar administration, the delta index again falls to a low level. Thus the delta index varies inversely to the alpha frequency and cerebral oxygen consumption.

The metabolic rate is only one of the modifiers of cerebral electrical activity. Lennox, Gibbs and Gibbs (15) have demonstrated that changes of the pH of the blood are accompanied by alterations of electrical frequency, alkalosis causing a diminution and acidosis an increase. The pH of the brain, however, does not necessarily follow that of the blood (Dusser de Barenne et al., 16). Since insulin acts predominantly on the brain, reducing metabolism by the removal of its chief substrate, it might be expected that acid intermediaries are produced in small amounts during hypoglycemia. This would tend to shift the pH of the cortex to the alkaline side as previously suggested by Georgi (17) and thus act synergistically with depressed cerebral metabolism to reduce the frequency of the brain waves.

SUMMARY AND CONCLUSIONS

1. Simultaneous observations were made of the electroencephalographic changes and oxygen utilization of the brain and of blood sugar levels of patients with schizophrenia receiving the insulin treatment.

2. A direct relationship is disclosed between the frequency of the alpha waves and the cerebral oxygen consumption, both declining during hypoglycemia and increasing after the administration of carbohydrate.

3. The delta index displays an inverse relationship for it is augmented during the period of diminished oxygen utilization of the brain.

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STUDIES ON EXPERIMENTAL HYPERTENSION IN THE RAT

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Since the kidney has assumed great importance in the study of experimental hypertension (1), a great deal of work has been done on this subject in laboratory animals. The dog has been the animal used in these studies, chiefly because the blood pressure can be followed in these animals without much difficulty. Comparatively little work on hypertension in smaller animals has been reported. This report deals with the physiological effects, in rats, of hypertension which was produced by subtotal nephrectomy, after the method of Chanuten and Ferris (2), as modified by Rytand and Dock (3). In many experiments subsequent bilateral adrenalectomy was performed with the aim of modifying the hypertensive effects, as was done by Goldblatt (4) in the case of hypertension in dogs.

In order to follow the arterial blood pressure changes by repeated determinations over weeks or months, the direct manometric method of Woodbury and Hamilton (5) and Hamilton et al. (6) were not found suitable. An indirect method (details of which will be described elsewhere) was devised for the purpose. The method is in essence a modification of a method discarded as unsatisfactory by Chanuten and Ferris, but found satisfactory by us after an adequate cuff had been designed. The latter consists of a thin rubber tube traversing a cone-shaped pressure chamber of glass. The tail of the anesthetized rat was inserted through this rubber tube, the circulation in the artery of the tail being interrupted by raising the pressure in the glass chamber by means of a pressure bulb; the pressures were read from a monometer as the circulation was reëstablished by gradual release of the constricting pressure. The tip of the tail beyond the constricting cuff was snipped off with scissors and immersed in 0.2 per cent sodium citrate to prevent coagulation. As the compressing pressure falls, the character of the stream of blood was noted to change at two points, the first of which may be called "systolic" and the second "diastolic," although it may be questioned whether these designations

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are strictly correct. The "first point" may be recognized by the appearance of jets of blood which look like beads as they fall through the citrate solution. The "second point" marks a sudden increase in the stream of blood which may be checked by increasing the cuff pressure or ligating the end of the tail. Readings are reproducible and, as may be noted in table 1, section A, compare favorably with the corresponding results obtained by Woodbury and Hamilton, who measured the blood pressure of rats by a direct method.

TABLE 1

Blood pressures of rats determined by indirect cuff-method and direct aortic needle puncture

DESCRIPTION OF EXPERIMENT	CONDITION OF RATS	NO. OF ANIMALS	INDIRECT CUFF METHOD						MEAN BLOOD PRESSURE CALCULATED FROM "FIRST POINT" AND "SECOND POINT"	DIRECT AORTIC NEEDLE METHOD		
			"First point"			"Second point"						
			Average	Minimum	Maximum	Average	Minimum	Maximum		Average	Minimum	Maximum
Section A												
Normal rats	Normal	53	118	90	140	66	40	95	92	110*	93	125
Section B												
Effect of subtotal nephrectomy on blood pressure	Normal	23	114	90	140	59	40	75	87			
	1 week following subtotal nephrectomy	23	149	120	190	85	50	110	117			
	2-3 months postoperative	23	201	150	250	108	70	140	155			
	3-4 months postoperative	23	225	190	270	131	100	170	177	176†	164	200
Section C												
Effect of adrenalectomy on hypertension produced by subtotal nephrectomy	Normal	12	105	95	140	64	50	90	85			
	3-4 months after subtotal nephrectomy	12	233	190	250	128	100	160	181			
	2-7 days after bilateral	12	149	130	190	74	60	85	112	116.4‡	105	130

* Determined in 11 rats.
† Determined in 5 rats.
‡ Determined in 9 rats.

Normal arterial pressure in rats. The pressures observed in the caudal artery were quite comparable with the aortic pressures of rats obtained by Durant (7) and Leiter (8) with a direct method. Williams, Weigriea and Harrison (9) found 94 per cent of the aortic pressures to fall between 90 and 140 mm. Hg. In Leiter's series, 69 per cent were between 101 and 140 mm. Hg and 20 per cent above 140 mm. Hg. In our experiments, we found 87 per cent of the "first point" pressures were between 101 and 130 mm. Hg and 90.6 per cent of the "second point" pressures

were between 41 and 80 mm. Hg, the variation in normal animals thus being less than those reported by Leiter.

The sensitivity of the method of registering the blood pressure was attested by the fact that epinephrine in two experiments raised the "first point" pressure from 160 to 210 mm. Hg in three to five minutes; while the "second point" rose from 90 to 140 mm. Hg. Thirteen minutes later the pressures were 180 and 120 mm. Hg, respectively.

Effects of partial nephrectomy. A. *Blood pressure changes.* Forty-one animals were subjected to partial nephrectomy, approximately five-sixths of both kidneys being removed. The operation was performed in two stages. Twenty-three animals survived and were observed over a period of three months or longer. All of these showed a progressive increase in arterial blood pressure as may be seen in section B of table 1. In five instances of marked hypertension, as determined by the tail cuff method, the aortic pressures were also determined by needle puncture and manometer and the presence of hypertension was confirmed.

The rise was considerable, even a few days after subtotal nephrectomy; it was marked at the end of two months, and still further increased at the end of three months. Rat number 35 will illustrate the average rise in arterial blood pressure; the normal blood pressure was 110/60 mm. Hg; after eight days it was 160/100 mm. Hg; after fifty-eight days it was 180/100 mm. Hg; after ninety-four days it was 235/150 mm. Hg.

B. *Weight changes of organs.* The effect of chronic experimental renal hypertension on the weights of the various organs is shown in figure 1. The weights of the endocrine glands are given in milligrams per 100 grams of body weight. The normal weights of these organs were given in a previous paper (10). Hypertension caused a slight decrease in the weights of the ovaries and thyroids, as compared with the organ weights of normal animals; there was no significant change in the weights of the pituitaries. The adrenals increased in weight slightly, while the kidneys and the hearts increased in weight markedly, as compared with the organ weights of normal animals.

C. *Histological changes.* Tissue for histological study was fixed in Bouin's solution and stained with hematoxylin and eosin. In so far as could be determined in the tissue so prepared, the pituitaries and the thyroids of hypertensive animals were essentially normal. The ovaries showed moderate atrophy. The uteri showed normal histological changes compatible with estrus or dioestrus. The adrenals of hypertensive animals showed changes suggestive of hyperplasia in the medulla, while the cortex seemed to be relatively thin. There was much vacuolization between the medulla and cortex. The kidneys of the hypertensive animals showed, in general, degenerative lesions resembling markedly

the picture seen in acute glomerulonephritis. Similar lesions have been described by Wood and Ethridge (11).

D. *Vaginal smears.* Vaginal smears of seven hypertensive animals were examined daily for four to six weeks. All showed a definite disturbance of the oestrous cycle. In some instances the vaginal smears were cornified throughout the period of observation; in other instances, the dioestrous interval was greatly prolonged.

Effect of adrenalectomy. Section C of table 1 illustrates the effects of bilateral adrenalectomy on the hypertension produced by partial

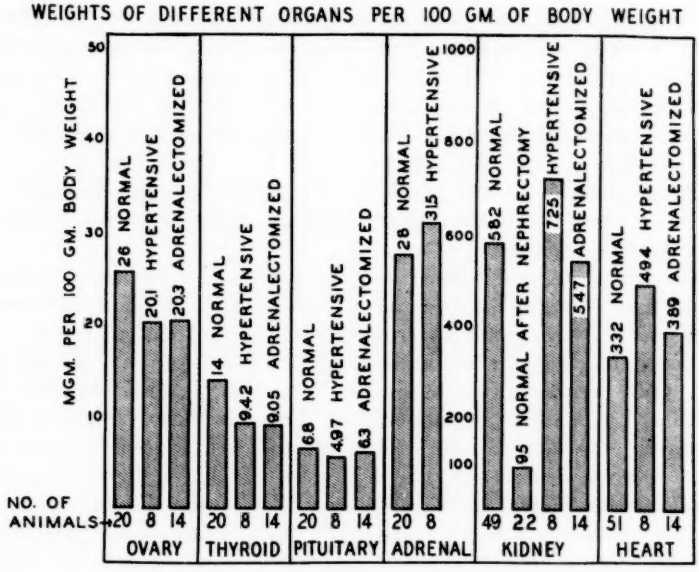

Fig. 1

nephrectomy in 12 rats. Blood pressures were determined two to seven days following removal of the adrenals. There was no apparent relationship between the height of blood pressure and the length of time after adrenalectomy. The animals were active and apparently remained in good condition. Two of the animals in which the blood pressures were determined 48 hours after operation received no supportive therapy. The remainder were given 10 cc. of normal saline subcutaneously each day. The "first point" pressures were reduced, on the average, by 67 mm. Hg, and the "second point" pressures by 54 mm. Hg, as compared with corresponding pre-adrenalectomy pressures. Both the "first point"

and "second point" pressures remained above the control (pre-adrenalectomy, hypertensive) level.

Two hypertensive rats, whose blood pressures showed marked reduction on the sixth day after adrenalectomy, were injected subcutaneously with adrenal cortical extract (Eschatin) daily for 6 days thereafter. At the end of this period there was a questionable elevation in the "first point" pressures, but no significant change in the "second point" pressures. The aortic pressures remained within normal limits.

Adrenalectomy of hypertensive animals did not cause any change in the weights of the ovaries, thyroids or pituitaries as compared with the organ weights of hypertensive animals; however, the weights of the kidneys and hearts of adrenalectomized animals were markedly reduced as compared with the corresponding organs of hypertensive animals. Histological sections of the pituitaries, thyroids and ovaries of these adrenalectomized animals showed nothing unusual as compared with the corresponding organs of hypertensive animals. However, there was a distinct difference in the renal lesions following adrenalectomy. In this case, there was focal tubular damage, together with evidence of old glomerulo-nephritis.

Discussion. A progressive increase in pressure over a period of several months occurred following partial nephrectomy. The effect of bilateral adrenalectomy on chronic experimental renal hypertension is of interest in connection with the experiments of Goldblatt (4) and Blalock and Levy (12), in which hypertension in dogs, due to renal ischemia, was abolished by bilateral adrenalectomy. The observation that the blood pressure of rats does not return to the control level (i.e., the level recorded before subtotal nephrectomy) is of interest. It is well known that complete adrenalectomy in adult rats is not easily accomplished, due to the presence of accessory cortical tissue (13). In the dog, on the other hand, accessory cortical tissue is rarely present. Therefore, it is not known whether the failure of the blood pressure to return to the control level in rats is due to incomplete adrenalectomy, or to some other factor. However, the marked reduction in the blood pressure following bilateral adrenalectomy (especially since these animals remained in good physical condition, showing no evidence of shock) can not be accounted for on any basis other than the removal of most, if not all, of the adrenal tissue.

The increase in weight of adrenals of hypertensive animals over the weight of adrenals of normal animals is small, but significant. This increase could be accounted for by the apparent hyperplasia and vacuolization found in the medulla.

The increase in weight of the kidneys and hearts of hypertensive animals has been commented upon by others. The reduction in weight of the kidneys and hearts following adrenalectomy is rather striking. The

decrease in weights of the hearts could be accounted for on the basis of lowered blood pressure, resulting in atrophy of the hearts. Generalized wasting of the body as a result of adrenalectomy may play a part. The reduction in weight of the kidneys following adrenalectomy is difficult to explain. The hypertensive kidney shows much edema, albumin in the tubules, cloudy swelling and endoarteritis; while the kidneys following adrenalectomy show principally focal changes in the tubules. The difference in weights may be due to the difference in water content of the tissues.

The decrease in the weight of ovaries of hypertensive animals is associated with histologic evidence of moderate atrophy, as well as by evidence of ovarian dysfunction (i.e., disturbances in oestrous cycle). It has long been a clinical impression that hypertension in the female is most common around the menopause, when there is ovarian failure. The effect of hypertension on the sexual apparatus is not clear. The present study affords an instance in which experimental hypertension has disturbed the normal function of the rat's ovary, as shown by the vaginal smears.

SUMMARY

An indirect method for obtaining repeated determinations of blood pressure in rats has been described briefly. The blood pressures obtained with the indirect method agree with those obtained by the direct method of aortic cannulation.

A progressive increase in blood pressure over a period of three to four months following partial nephrectomy has been observed. This type of experimental hypertension has been markedly reduced following bilateral adrenalectomy. Thus the results of the present experiments on rats are similar to those obtained by Goldblatt after adrenalectomy in hypertensive dogs, the hypertension having been produced by renal ischemia.

The weights of various organs of hypertensive rats have been tabulated. A slight increase in the weight of the adrenals in hypertensive rats has been observed. Changes in the weights of kidneys and hearts in hypertensive animals, similar to those described previously by Rytand and Dock, and Chanuten, have been observed. A reduction in the kidney and heart weights following adrenalectomy of hypertensive animals was noted.

The histological pictures presented by the adrenals of hypertensive animals and those of the kidneys following adrenalectomy of hypertensive rats have been described.

Hypertension produced by subtotal nephrectomy has produced a definite disturbance in the sexual cycle of rats, indicating an ovarian dysfunction.

We wish to express our deep appreciation to Dr. J. C. Burch and Dr. Alfred Blalock for the facilities supplied and for their advice throughout the work.

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VITAMIN E DESTRUCTION BY RANCID FATS[1,2]

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The destruction of vitamin E by fats subjected to slight oxidation has been demonstrated (Mattill, 1927, and Evans and Burr, 1927a). Olcott and Mattill (1934) showed that destruction of the vitamin E-activity of cottonseed oil concentrates was effected by oxidation with $KMnO_4$ and by oxygen containing 10 per cent of ozone in a petroleum ether solution. So far, however, the effect of fats of a measured degree of rancidity or of isolated products of rancidity has not been determined. The experiments reported in this paper include results on the effect of rancid fats of known peroxide value and of various aldehydes, ketones, and other possible decomposition products which may be present in rancid fats.

EXPERIMENTAL. Our first objective was the production of rats which showed consistently the typical symptoms of vitamin E deficiency as described by Evans and Burr (1927b). Young females were placed at weaning (40 to 50 grams) on a ration essentially the same as that of Olcott and Mattill (1934) composed of crude casein 18, brewer's yeast[3] 8, sucrose 37, salts[4] 5, fat 30, and cod liver oil[5] 2 per cent. The animals were mated 10 to 12 weeks after weaning at a weight of 200 grams. Daily examinations of the vaginal smears were made to detect the presence of sperm cells, and later the "placental sign." Daily weighings were made throughout gestation. Animals which resorbed, lost weight during the last five days of pregnancy with reappearance of the oestrous cycle from the 20th to the 25th day.

Lard and a hydrogenated vegetable shortening (fat A)[6] were used as

[1] The authors wish to express their appreciation to Lever Brothers Company whose financial support made this research possible.

[2] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station, Madison.

[3] Anheuser-Busch.

[4] Osborne and Mendel (1917) as modified by Hawk and Oser (1931) with omission of the sodium fluoride.

[5] Mead Johnson.

[6] The fats used were those available commercially. Hydrogenated shortening A was manufactured by hydrogenating cottonseed oil to an iodine number of approximately 70. The saponification number was 196.0.

the fat ingredients of the ration. When rancid fats were required they were produced from these by aeration as follows: Three flasks, each containing 750 cc. of the fat, were connected in series and aerated rapidly with moist air. Each flask was heated and illuminated by two 200-watt Mazda bulbs. The development of rancidity was followed by occasional determinations of peroxide numbers expressed as millimoles of active oxygen per kilogram of fat. To minimize changes in rancidity of the fats after incorporation in the rations, the rations were made up weekly and stored in a refrigerator at 4°C.

A decided difference was soon apparent between the rats reared on fat A and on the fresh lard rations. While litters were always obtained on the hydrogenated fat, only 8 litters were obtained from 750 rats on the fresh lard, yet these animals were sleek and active and grew on the average 14 grams per week in 10 weeks. This low incidence of initial fertility is entirely in accord with results reported by Olcott and Mattill (1937) and by Bacharach, Allchorne and Glynn (1937).

It was a question if lard was directly responsible for the resorptions or if it exerted a deleterious effect on the vitamin E activity of other ingredients of the ration. The lard was accordingly omitted entirely from some rations with substitution of 30 per cent sucrose or glycerol, or reduced to levels as low as 5 per cent with corresponding increases in the sucrose content of the ration. Resorptions were still obtained regardless of how much the percentage of lard had been reduced, although apparently not so consistently. From 22 animals 3 litters were obtained with the first pregnancy and none with the second. However, the animals on 5 per cent or less of lard did not grow quite so well nor appear so sleek and well-nourished as those on 30 per cent. The 30 per cent fresh lard diet was therefore selected as the most suitable for the production of vitamin E-deficient animals.

It was not found necessary to use rancid lard to secure resorptions. As a matter of fact highly rancid lard (peroxide no. 200) in the ration reduced consumption, and the animals increased in weight on an average of 12.3 grams in the first 10 weeks, instead of 14.0 grams with fresh lard. In addition, the rancid lard diet seemed to affect subsequent pregnancies. For example, in a group of 5 animals 2 resorptions occurred in a second pregnancy although 30 per cent of fat A had been substituted for the lard. Later experiments showed that only 5 per cent of fat A was necessary to produce a successful pregnancy in animals reared on a fresh lard diet.

Animals reared on the ration containing 30 per cent of fresh lard were mated at a weight of 200 grams and proven to be vitamin E-deficient by the incidence of resorptions. The animals were then divided into groups of 6 to 8 animals each and mated again. The diets to be tested were given throughout pregnancy and the incidence of litters or resorptions noted. In

all cases the test diets were identical with the basal vitamin E-low diet, except for variation in the fat content as indicated. Ad libitum consumption of the rations was allowed.

In some instances an animal which had resorbed on a test diet was used for a second test. However, it was found inadvisable to attempt to use an animal for more than 2 test periods. Failure of implantation after successful mating increased in frequency with successive resorptions. When an attempt was made to use a group of 26 animals in a third test period, failure to secure implantation became a serious problem. Sixty per cent of the group failed to become pregnant. A subsequent examination of the records revealed that no failures had occurred in the initial pregnancy or in the first test period, but an incidence of 19.2 per cent was noted in the second test period. Out of 253 animals whose records were scanned, 2.2 per cent failed in the initial gestation, and 8.0 and 10.8 per cent of 157 animals in the first and second test periods, respectively. Comparable difficulties have lately been reported by Palmer (1937) and by Bacharach, Allchorne and Glynn (1937).

Source of vitamin E. Fat A was used as a standard by which the destructive effect of rancidity was measured for several reasons. Because of hydrogenation and the presence of natural antioxidants, it was resistant to oxidation at temperatures encountered in the laboratory, yet by aeration at increased temperatures and exposure to light it could be made rancid at will. Added favorable features were its ready availability, its uniformity in composition, and its relatively high and stable content of vitamin E. A sample stored in the refrigerator retained its activity unimpaired for 5 years.

Vitamin E-deficient rats were placed on test diets containing 30 per cent or less of fat A with the total fat content equalized at a 30 per cent level by the further addition of fresh lard; litters were obtained in all cases (table 1). Litters were always obtained with 5 per cent of fat A as the source of vitamin E. On lower levels, namely, 1.0 and 0.2 per cent, only 2 litters were obtained from 12 females. An addition of 5 per cent fat A to the basal diet was used for positive controls. In all, 31 rats on the positive control diet gave 29 litters with a total of 159 young. Two females died during parturition. The litters were sometimes small and occasionally the young were born dead, but resorption of an entire pregnancy was never obtained. When a new series of experiments was started, 5 or 6 animals were always kept on the basal diet as negative controls.

Effect of fats made rancid by aeration. Fresh lard of a peroxide number of approximately 1 was aerated as described above to peroxide numbers of 4, 11, 27, and 50 respectively. The resultant fats were incorporated at a 25 per cent level in the basal diet which contained 5 per cent of fat A. Litters were produced with lard of a peroxide number of 4, but not 11

TABLE 1

Effect of rancid fats and fat decomposition products on the vitamin E activity of a fresh fat

FAT OR FAT DECOMPOSITION PRODUCT IN TEST DIET	ANIMALS IN GROUP	LITTERS BORN	YOUNG FOUND*	RESORPTIONS
30% fresh fat A	4	4	22‡	
20% fresh fat A, 10% fresh lard	7	7	46	
10% fresh fat A, 20% fresh lard	5	5	30	
5% fresh fat A, 25% fresh lard	10	9†	58	
1% fresh fat A, 29% fresh lard	6	2	9	4
0.2% fresh fat A, 29.8% fresh lard	6			6
5% fresh fat A				
25% aerated lard (per. no. 4)	6	5†	19	
25% aerated lard (per. no. 11)	6			6
15% aerated lard (per. no. 11), 10% fresh lard	6	6	29	
25% aerated lard (per. no. 27)	7			7
25% aerated lard (per. no. 50)	6			6
30% aerated fat A (per. no. 11)	5	4	21	1
5% fresh fat A				
25% aerated fat A (per. no. 11)	6	6	40	
25% aerated fat A (per. no. 25)	7	6†	34	
25% aerated fat A (per. no. 50)	7			7
5% fresh fat A				
25% ozonized lard (per. no. 11)	5			5
25% fresh lard and palmitic peroxide (per. no. 1440) mixed to a per. no. of 11	6			6
25% fresh lard and aerated lard (per. no. 187) mixed to a per. no. of 11	6	6	29	
25% fresh lard and solarized fat A (per. no. 191) mixed to a per. no. of 11	5	3	16	2
25% fresh lard and solarized lard (per. no. 127) mixed to a per. no. of 11	6	6	37	
25% aerated lard (per. no. 163) heated to a per. no. of 11	5	2	8	3
0.5% wheat germ oil, 30% fresh lard	6			6
1.0% wheat germ oil, 30% fresh lard	7	6	31	1
1.0% wheat germ oil, 30% aerated lard (per. no. 30–35)	6			6

* Occasionally the adult rat consumed part or all of the young before they were counted. That a litter had been born was determined by drop in weight of the adult on the 22nd day of gestation, by the presence of blood on the shavings, or by the finding of partly consumed carcasses in the cage.

† One female died at parturition.

‡ From 3.

TABLE 1—*Concluded*

FAT OR FAT DECOMPOSITION PRODUCT IN TEST DIET	ANIMALS IN GROUP	LITTERS BORN	YOUNG FOUND*	RESORPTIONS
2.0% wheat germ oil, 30% aerated lard (per. no. 30–35)	8			8
3.0% wheat germ oil, 30% aerated lard (per. no. 30–35)	7			7
0.5 gm. fat A daily on empty tract 30% fresh lard diet 6 hrs. later	7	7	30	
0.7 gm. fat A daily on empty tract 30% fresh lard diet 6 hrs. later	8	8	49	
0.5 gm. fat A daily on empty tract 30% rancid lard (per. no. 30–35) ration 6 hrs. later	8	8	42	
0.7 gm. fat A daily on empty tract 30% rancid lard (per. no. 30–35) ration 6 hrs. later	7	7	44	
30% fat A heated in an open pan at 150°C. for 1 hr., (per. no. 19.2)	6			6
30% fat A heated in an open pan at 125°C. for 1 hr., (per. no. 7.8)	2	2	7	
30% fat A heated in an open pan at 100°C. for 1 hr., (per. no. 4.0)	5	5	21*§	
5% fresh fat A, 25% fat A heated in an open pan, 150°C. for 1 hr., (per. no. 19.2)	4	2	2	2
30% fat A heated under nitrogen reflux, 185°C., 1½ hrs. (per. no. 1)	7	7	42	
30% fat A heated under nitrogen reflux, 150°C., 1 hr. (per. no. 0.8)	6	6	29	

§ From 4.

or higher. When the amount of lard with a peroxide number of 11 was reduced to 15 per cent with the addition of 10 per cent of fresh lard, litters were again secured.

Similarly fat A itself, of a peroxide number of 0.9, was made rancid to peroxide numbers of 11, 25 and 50 respectively, and fed at a 25 per cent level in rations containing 5 per cent of the fresh fat. Litters were obtained with fat of a peroxide number of 25, but not at 50. Because rancidity produced in fat A had to destroy the vitamin E activity of the fat itself as well as that of the added fresh fat, one diet containing 30 per cent of fat A of a peroxide number of 11 was included in the series. Enough residual vitamin E activity was found in this rancid fat to result in the production of 4 litters from 5 pregnancies. However, in one litter parturition was delayed 3 days beyond the usual 22nd day of gestation and then only 1 young was born. It is probably safe to assume that fat A

made rancid to peroxide numbers greater than 11 contained little residual vitamin E, but destruction of added vitamin E did not occur until a peroxide number of 25 to 50 had been attained.

Effect of various lards. Because aerated lard of a peroxide value of 11 destroyed vitamin E, other samples of lard were brought to similar peroxide values for a determination of their destructive action. This was done by the addition of palmitic peroxide, by aeration with ozonized oxygen, by heating an aerated lard of a peroxide value of 163 under nitrogen and by diluting lards made rancid by aeration or exposure to sunlight with the required amount of fresh lard. The results show that ozone and palmitic peroxide always destroyed vitamin E but that other methods of treatment were not always so effective,—litters were produced in many cases.

Effect of a given rancid fat on increasing amounts of vitamin E. An attempt was made to overcome the destructive effect of a moderately rancid fat by progressively increasing the amount of vitamin E in the diet.

Fresh lard was compared with a lard of a peroxide number of 30 to 35 as prepared by aeration. These were fed at 30 per cent levels with 0.5 to 3 per cent of wheat germ oil[7] incorporated at the expense of an equal amount of sucrose. Wheat germ oil was selected as the source of vitamin E because its high potency made it possible to administer a fairly large amount of vitamin E without a great increase in the amount of fat in the ration. Lard of a peroxide number of 30 to 35 was used because it was moderately rancid, yet sufficiently palatable. Food consumption in the test diets was limited to 8 grams per animal per day. As pregnancy advanced, extra food was given in the form of the basal diet with either 30 per cent fresh fat or rancid fat depending on what the rats had received before.

With one exception litters were obtained on the diet containing 1 per cent of wheat germ oil and fresh lard, but at the lower level of 0.5 per cent of wheat germ oil, resorptions occurred. Complete resorption was obtained in all animals on the rancid fat, even when wheat germ oil was incorporated at a 3 per cent level. It is unfortunate that a higher level of wheat germ oil was not fed, but it can be concluded that 30 per cent of a moderately rancid lard is able to destroy at least 3 times the amount of vitamin E which would produce litters on a fresh lard diet.

Effect of vitamin E and rancid fat administered separately. To demonstrate that the rancid fat must be mixed with the vitamin E to effect destruction, these constituents were fed separately with an intervening period of 6 hours. Female rats which had been depleted of vitamin E were fasted over night (13 hours) and then given measured amounts of melted fat A with a small amount of the dry ingredients of the basal

[7] Abbott Laboratories.

ration at 8 o'clock each morning. Six hours later they were given the vitamin E-low basal ration containing rancid lard of a peroxide number of 30 to 35. After 5 hours' feeding, the 13-hour fast was again imposed. This routine was repeated daily, from the first to the twentieth day of gestation.

Fat A was fed at two levels, namely, 0.5 and 0.7 gram per day, measured out volumetrically with 1 cc. tuberculin syringes. A total of 10 or 14 grams was administered to each animal during gestation. These amounts fell within the range of the quantities consumed with 5 per cent of fat A in the diet. The reproductive performance of the animals receiving the rancid lard diet was as good as those receiving the fresh lard. Apparently no destruction of vitamin E resulted.

Effect of decomposition products of fats. Because palmitic peroxide and ozonized lard, as well as rancid fats, destroyed vitamin E, decomposition products of fatty acids and glycerol, namely, alcohols, aldehydes and ketones were also tested. A week previous to feeding, fat A equivalent to 5 per cent of the final diet was weighed out, barely heated to melting, and mixed with the decomposition product desired. Allyl alcohol and acrolein, respectively, were incorporated at levels of 0.05 per cent of the final ration. Mixture of aldehydes[8] and of methyl ketones were tested at 1 per cent levels. These mixtures were made by taking equal amounts of the straight-chain compounds from C_7 to C_{12} inclusive.

The mixtures of decomposition products with fat A were allowed to stand 1 week at 4°C. to give ample time for destruction of vitamin E without the development of rancidity. Enough fresh lard was then added to bring the fat content of the final diet up to 30 per cent; then 2 per cent of cod liver oil and the dry ingredients of the ration were incorporated. Little, if any, destruction occurred under the conditions imposed. Each diet was tested on a group of 4 to 5 animals and litters were obtained with all pregnant females except one.

Effect of fats made rancid by heating. It was observed that fat A heated to 150°C. at a depth of 1 cm. in an open pan 6 inches in diameter acquired a maximum peroxide number of 19.2 in 1 hour. When heated above 150°C. the peroxides were decomposed and below this temperature they were not produced in quantity.

When 30 per cent of fat which had been heated at 125°C. and 100°C., with resultant peroxide numbers of 7.8 and 4.0 respectively, was fed to rats, litters were obtained; but with one heated at 150°C., complete resorption resulted. It was impossible to say, however, that no destruction took place at the two lower temperatures for fat A without treatment at this level introduced 6 times the amount of vitamin E necessary to produce

[8] Obtained from Lever Brothers Company.

normal litters. When heated in the absence of air, that is in nitrogen, no destruction of vitamin E activity occurred.

SUMMARY

1. In the standard procedure for rearing vitamin E-deficient animals, a diet similar to that used by Olcott and Mattill (1934) was adopted. Initial fertility was encountered in only 8 out of 750 animals so reared.

2. The failure of implantation after positive mating was a difficulty encountered with increasing frequency in successive resorptions. This failure did not reach annoying proportions until the animals were subjected to a third test period after the initial resorption.

3. A partially hydrogenated vegetable shortening, incorporated at a 5 per cent level in an otherwise vitamin E-deficient ration, restored fertility in female rats whose previous gestations had ended in resorption. This activity was destroyed by certain fats made rancid by aeration, by heat in the presence of oxygen, by treatment with ozone, or by addition of palmitic peroxide. No destruction was observed with fats heated under nitrogen reflux, nor with fats to which acrolein, allyl alcohol, or straight-chain aldehydes and ketones had been added.

4. When a moderately rancid fat and a source of vitamin E were administered separately to avoid a possibility of reaction in the ration and in the gastro-intestinal tract, no destruction of the vitamin occurred,—contact in the ration was shown to bring about destruction.

5. A three-fold increase in the amount of vitamin E necessary to produce litters on a fresh fat diet did not overcome the destructive action of a fat made moderately rancid by aeration.

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THE RESPIRATORY METABOLISM OF INFANT BRAIN[1]

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The work of numerous investigators has shown that the brain *in situ* of adult animals oxidizes carbohydrate predominantly (1, 2). Moreover, *in vitro* studies sustain the view that carbohydrate is the chief foodstuff of the brain (3, 4, 5). On the other hand, the problem of the foodstuffs used by that organ to satisfy its energy requirements during early postnatal development has received but little attention. The present study was undertaken, therefore, to determine whether or not brain excised from immature animals shows the same substrate preference as adult brain, and to ascertain whether the enzyme systems are present at birth or develop as the animal matures.

METHOD. The infant rats used for this study (from 1 to 24 days old) were obtained from the colony of the Department of Biochemistry, Albany Medical College.[2] Since the amount of cerebral tissue available in an infant brain is small, both cerebral hemispheres were used, and the tissue of several rats from the same litter was taken for a single observation. The use of the entire cerebral hemispheres is warranted for the brain of infant rats contains little lipoid material. The Dixon-Keilin manometric method was employed to determine the QO_2 (mm.^{3} oxygen absorbed per mgm. dry weight of tissue per hour), the respiratory quotient and the aerobic glycolysis, QA, (mm.^{3} carbon dioxide evolved, equivalent to lactic acid, per mgm. dry weight of tissue per hour) of *sliced* cerebral tissue suspended in Ringer-Krebs medium (6) containing bicarbonate, in the presence of 0.02 M glucose or lactate. Additional experiments with minced whole brain of infants and minced cerebral gray matter of adult rats were done, using the Warburg manometer. The mince was suspended in phosphate-saline (pH = 7.4) with the same concentration of substrate as used in the experiments with sliced cerebral cortex. Respiratory quotients and oxygen consumption were determined.

[1] This investigation was aided by a grant from the Child Neurology Research (Friedsam Foundation).

[2] Thanks to the courtesy of Dr. Arthur Knudson.

RESULTS. Table 1 presents the average values for QO_2 and R.Q., and their standard deviations obtained in 137 experiments with sliced cerebral cortex[3] of rats, 1 to 11 days old and 12 to 24 days old, without added substrate, and in the presence of glucose and lactate. The values in the absence of substrate and with lactate are not unlike those of the adult in terms of dry weight. The QO_2 in the absence of substrate averages 5.9, and is raised by the addition of lactate to an average value of 13.2 which is very close to the values obtained on adult rats: 6.3, and 13.3 with no

TABLE 1

Average oxygen consumption of infant sliced cerebral hemisphere

AGE	NO SUBSTRATE		+ GLUCOSE		+ LACTATE	
	$-QO_2$	R.Q.	$-QO_2$	R.Q.	$-QO_2$	R.Q.
days						
1–11	5.9 ±0.16	0.96 ±0.02	11.7 ±0.17	0.96 ±0.01	13.2 ±0.28	0.95 ±0.01
12–24	5.9 ±0.18	0.92 ±0.02	13.6 ±0.27	0.90 ±0.01	13.1 ±0.31	0.91 ±0.01

TABLE 2

Oxygen uptake (100 mgm. wet weight per hour) of minced cerebral tissue of the brain of three infants (7 days old) and three adult rats

OXYGEN CONSUMPTION MM.[3]	
Infant	Adult
141	206
126	216
114	201
119	189
118	189
121	200
124	216
118	181
	205

added substrate and lactic acid respectively. The observations with glucose on infant brain, however, reveal an average QO_2 from 1 to 11 days of 11.7 and from 12 to 24 days of 13.6, a significant difference. When, however, the oxygen uptake is calculated in terms of original wet weight for minced brain (mm.[3] per 100 mgm. moist tissue per hour), the infant tissue is found to possess an oxygen uptake, which is significantly smaller than the adult, 123 compared to 200 (table 2). To summarize the results of tables 1 and 2: oxidations, in general, proceed more slowly in the infant

[3] Young rats—entire cerebral hemispheres, older rats—gray matter.

brain than in the adult; moreover, in the first 11 days glucose is less rapidly oxidized than lactic acid, although in the adult brain both are oxidized at approximately the same rate. Aerobic glycolysis, on the other hand, is practically the same throughout life, the values for the infant brain (QA) averaging −1.6, a value not significantly different from the adult.

TABLE 3

Oxygen uptake (100 mgm. wet weight per hour) of minced cerebral tissue of the brain of newborn rats

	WITHOUT ADDED SUBSTRATE O_2 UPTAKE MM.^{3}	WITH SUBSTRATE O_2 UPTAKE MM.^{3}	R.Q.
	65	94	0.58
	70	89	0.55
	73	93	0.40
	33	82	0.45
Succinate	40	79	0.51
	37	64	0.46
	60	116	0.48
	75	135	0.59
		125	0.47
	66	77	1.15
	63	77	1.15
		77	1.12
Pyruvate			
	38	53	1.09
	42	58	1.11
		53	1.16
	46	66	0.83
	46	71	0.80
	56	69	0.80
Fructose			
	50	80	0.83
	50	80	0.80
	48	82	0.80

As seen in table 3 succinate, pyruvate, and fructose increase the oxygen uptake of minced infant brain. Succinate and pyruvate tend to produce characteristic changes on respiratory quotient, succinate lowers the quotient while pyruvate raises it. Fructose has little effect on the respiratory quotient and in this regard behaves like glucose in the presence of minced infant brain. Numerous observations made on *minced* cerebral tissue in the Warburg manometer revealed a lower R.Q. either with glucose or without added substrate than that obtained with *sliced* tissue. Since

mincing involves injury to a larger number of cells, and presumably their enzyme systems, than does slicing, it is probable that the lower R.Q. may be attributed to incomplete oxidations. Moreover, the technique of calculating R.Q.'s from data obtained on minced tissue on a wet weight basis is open to criticism. We, therefore, believe that the significant R.Q.'s are those obtained with sliced tissues.

Discussion. The data presented in this paper indicate that the respiratory processes of infant brain, like adult brain, are dependent on added carbohydrate. The low cerebral QO_2 of the infant in the absence of substrate, which can be doubled by the addition of glucose or lactic acid, emphasizes the importance of carbohydrate in the maintenance of cerebral metabolism and suggests that the intrinsic fat is not directly concerned with the energetic requirements. Further evidence for this is found in the high R.Q. in the absence of added substrate, both before and after myelinization takes place; a process which occurs about the 15th day in the rat. The respiratory quotient is not changed by the addition of glucose or lactate. This is at least suggestive that tissue carbohydrate is oxidized in the absence of added substrate.

Qualitatively, the same foodstuff is used but quantitatively a significant difference is observed, for the infant rat brain possesses a metabolic rate lower than that of the adult. It is true that per milligram dry weight of tissue the results obtained with infant and adult brain are similar, although in the presence of glucose, the younger rat tissues respire at a lower rate than the older. However, when these data are considered in terms of original wet weight, the metabolic rate of the adult brain is considerably higher, for the percentage of dry weight is greater in the older animals. At birth the dry weight of the brain is approximately 12 per cent of the wet weight, 19 per cent at 24 days, and 22 per cent in the mature rat (7). Thus the metabolism in terms of wet weight rises rapidly during the first three weeks of life, and the metabolism of the rat brain at 24 days has risen almost to the adult level. It is probable that per 100 grams of tissue in the living animal, the infant rat brain also exhibits a lower metabolism than the adult. For this reason, it becomes important to evaluate this phenomenon, and to consider possible causes and results for these differences. A consideration of the low oxygen uptake of the infant brain necessarily involves the respiratory enzyme systems concerned in these processes. As a first step toward an understanding of this problem, it may be well to examine the constituents of the cell in order to determine to which of these the low enzymatic activity of the infant brain may be referred. It is interesting to recall that in the adult the brain contains greater proportion of gray (protein) and white (lipoid) matter. It is not likely that the higher metabolism of the adult can be attributed directly to its lipoid contents since the white matter possesses a compara-

tively low metabolic rate (8). The greater rate of the adult brain, moreover, correlates with the larger protein content of the cells.

Koch and Koch (9, 10) have found that the increase of protein per milligram per day is 4.53 for 1 to 10 days and 5.9 from 10 to 20 days, and then falls abruptly to 0.89 from 20 to 40 days. The reason for this rapid increase in brain protein content probably lies in the fact that the newborn rat is physiologically young, younger, for example, than the neonatal pig and has been, therefore, rightly compared with the 50 to 100 mm. pig embryo (11). Multiplication of brain cells continues for some time after birth. More important, a marked growth of cells increases the total amount of protein of the brain. That a relationship exists between the protein content and the metabolism of an organism is well known. The low protein content of the infant brain is suggestive, therefore, that the difference between the infant and adult metabolism is not due to the development of new enzyme systems in the adult but rather to an increased concentration of those already present. This conclusion is in accord with the ability of infant cerebral tissue to oxidize the same substrates (glucose, fructose, lactate, pyruvate, and succinate) but at a slower rate.

The decreased metabolism is more marked for glucose than lactic acid. Although adult rat cerebral tissue oxidizes glucose and lactic acid at approximately the same rate, during the first 11 days of life the oxygen consumption in the presence of glucose is lower than that of lactic acid. Since the small values for aerobic glycolysis for the infant brain show no significant variations from the adult, the different rates of utilization of glucose and lactic acid cannot be attributed to any profound difference in the rate of aerobic glycolysis. The enzymes of oxidation, therefore, are the ones chiefly concerned. The present evidence indicates that glucose and lactic acid are oxidized at different rates. Results with nicotine (12) and hydroxymalonate (13) lead one to conclude that two different enzyme systems are involved in the oxidations of glucose and lactic acid. The fact that during earlier development lactate is oxidized more rapidly than glucose yields additional evidence for the concept that there are different paths of oxidation of glucose, one of these not involving lactic acid.

SUMMARY AND CONCLUSIONS

Studies of the respiratory metabolism of the brain of infant rats (1–24 days old) have been made. The following results have been obtained:

1. Like the adult brain, the infant is dependent on added carbohydrate (glucose) for maximum respiration and possesses a small aerobic glycolysis. The respiration of the infant brain is also stimulated by lactate, succinate, pyruvate, and fructose.

2. On the basis of *dry* weight, during the first 11 neonatal days, the rate

of glucose oxidation is lower than that of lactate. In the absence of substrate and the presence of lactic acid the respiration is the same as that in the adult.

3. The oxygen consumption of the brain of the newborn rat is smaller than that of the adult brain per unit of *wet* weight of tissue.

4. This diminished capacity for oxidation may be correlated with the protein concentration which is lower in the infant brain. It is, therefore, suggested that the diminished oxidation is a quantitative rather than a qualitative phenomenon.

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SPLENIC VOLUME AND THE POLYCYTHEMIA OF ARTIFICIAL FEVER IN INTACT, UNANESTHETIZED DOGS

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Many authors have described the polycythemia occurring in dogs during experimental artificial fever (1, 2, 4). An increase in the formed elements and plasma concentration of blood has been reported in both experimental infectious fever and in fever induced with high frequency currents (3). The mechanism for this polycythemia is generally explained as a concentration of blood owing to surface loss of water (chiefly from panting). Most of the experiments have been conducted over long periods of time and under conditions of anesthesia which might have influenced the concentration of blood elements. The studies about to be described were made in an effort to ascertain the true blood picture in intact, unanesthetized dogs with artificial fever of short duration, and to determine as far as possible the physiological processes involved.

Banus and Ginsburg (4), in experiments with infectious fever, noted a much greater increase in the formed elements than could be explained by a concentration of plasma. They suggested that this increase might be due to a liberation of red cells from a storage reservoir, possibly the spleen, as well as to surface loss of water. Barcroft and Elliot (3), using the exteriorized spleen technique, found that when dogs were placed in a heated chamber (47—54.5°C.) the normal spleen contracted (15 per cent) and the denervated spleen dilated (3–11 per cent). Further, a steadily increasing amount of evidence points to the fact that contraction or dilatation of the spleen under natural stimuli or drug action can cause highly significant changes in the red cell count of peripheral blood. Von Haam (6) demonstrated that normal dogs, when subjected to intravenous epinephrine injections, showed an acute polycythemia; splenectomized dogs did not. Recently, Hausner, Essex and Mann (7) showed that the spleen contracts with ether anesthesia, causing an increase as high as 1,800,000 per cu. mm. in red cells in the peripheral circulation; with amytal and the barbiturates it dilates, causing decreases as much as 2,5000,000 per cu. mm.

The above investigators, therefore, have demonstrated (1) that polycythemia occurs as a result of artificial fever, and (2) that splenic volume varies enough to cause concentration of formed elements in the periph-

eral circulation. The following investigation is an attempt to relate the above facts so as to determine whether or not the splenic volume decrease is the principal cause of the changes noted.

PROCEDURE AND METHODS. Artificial fever was induced in dogs by means of a General Electric Inductotherm.[1] This instrument was found to be ideally suited to animal experimentation, because the temperature could be readily controlled and the apparatus easily applied.

Continuous spleen volume records were obtained on intact, unanesthetized dogs under normal and artificial fever conditions using the method previously described (12). Dogs with chronic, subcutaneous spleens, prepared as described, were given the inductotherm treatment. A water manometer attached to a sphygmomanometer cuff wrapped around the spleen of the animal simultaneously recorded changes in spleen volume on a kymograph drum. In animals thus prepared, induction of fever caused a significant contraction of the spleen.

A series of normal animals were then subjected to treatment and blood studies were made. After the dog, trained to lie quietly, had been placed on a table, 30 minutes were allowed for adjustment to the new environment (thus ruling out emotional and exercise effects on the spleen). A sample of normal blood (10 cc.) was then drawn from the saphenous vein. The rectal temperature was taken, and fever produced by means of the inductotherm. This procedure was repeated after half an hour and again after a full hour of fever treatment. The machine was turned off, and, after another 30 minutes, the last sample was procured. The temperature increases varied slightly in different experiments, but the range of increase was always from 2 to 4° Fahrenheit.

To determine whether the resulting polycythemia was absolute or relative, red cell counts, hematocrit readings, and serum protein determinations were made on the blood samples. A few cubic centimeters of blood were placed in a small heparin tube, and red cell counts as well as hematocrit readings (Wintrobe) were made from the sample. The remainder of the blood was placed in a centrifuge tube and serum obtained for protein determinations. These were made by the total nitrogen method of Van Slyke with the Koch-McMeekin modification (8, 9).

After a series of experiments on a given dog, the dog was splenectomized, and the same determinations were made with the same procedures in another series of experiments after recovery of the animal (table 1).

RESULTS. Figure 1 shows the composite results of four normal dogs given fever treatments as above described. With the onset of artificial fever, there were significant increases in red cell count, hematocrit (cell

[1] I am indebted to Mr. H. J. Holmquist of the G. E. X-Ray Corporation for the use of the inductotherm.

volume), and serum proteins. In a typical case, the red cell counts increased over the normal by 25 per cent, the hematocrit by 14.5 per cent, and the serum protein by 12.5 per cent. Similar changes occurred in all dogs studied, though the amount of increase varied from animal to animal.

From these results alone, it would appear that both an absolute *and* a relative increase in the formed elements of the blood had occurred. Concentration of blood by surface loss of water (panting of the animal) would

TABLE 1

Four experiments on dog 4, showing actual data obtained from the normal animal—and from the same animal after splenectomy

DATE	TIME	T°F.	HEMATOCRIT	RBC	SERUM PROTEIN
		Normal			
1938			*cell vol.*	*millions*	*gr./100 cc.*
January 24	10:50*	101.4	34.0	5.110	5.78
	11:20	102.6	38.0	5.070	5.81
	11:50†	103.8	41.2	6.990	6.38
	12:20	101.8	37.0	4.990	5.77
January 31	9:50*	99.9	29.2	4.260	5.53
	10:20	102.4	35.6	4.925	6.14
	10:50†	103.7	40.0	5.410	6.80
	11:20	101.7	34.5	4.830	6.03
		Splenectomized			
February 16	9:40	100.4	36.0	4.940	6.35
	10:10*	102.0	36.2	5.420	6.41
	10:40	103.0	36.0	5.000	6.25
	11:10†	101.0	36.3	4.805	5.69
February 23	10:30	100.6	33.9	4.995	5.66
	11:00*	101.6	33.8	5.090	5.56
	11:30	102.5	35.4	5.220	5.81
	12:30†	101.3	35.2	4.945	5.80

* Inductotherm turned on after first sample.
† Inductotherm turned off after third sample.

explain that part of the increase in red cells which might be correlated with increase in serum concentration. The increase of R. B. C. above the level of serum concentration might be due to an influx of red cells from a storage reservoir in the animal. But in the splenectomized animals there was no significant change in the formed elements, and surprisingly, no change in the concentration of plasma. Thus it would seem that in these short duration experiments the surface loss is negligible and the all-

important factor is the liberation into the general circulation of red cells from the spleen.

There is still to be explained the increase of serum proteins in the normal animals. It is impossible to assume that this increase is due to a concentration factor, because the splenectomized animals behaved in the same way (panting) as did the normal animals. Two possibilities present themselves: 1, that the spleen liberates serum with concentrated protein together with the formed elements, and 2, that the increased red cell content in the circulation (due to contraction of the spleen) in some way causes a concentration of serum.

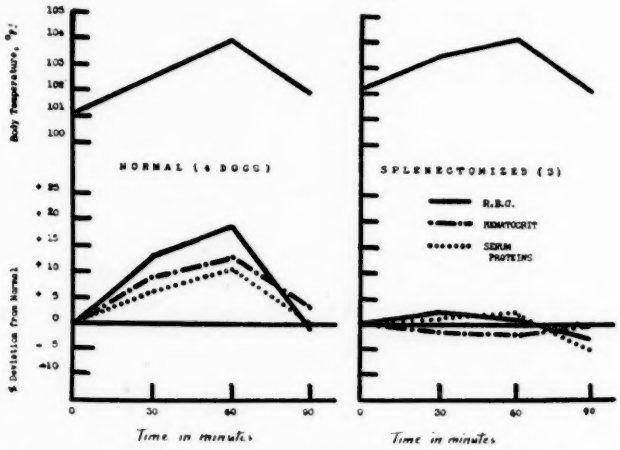

Fig. 1. Composite results of the effect of artificial fever treatment on the red cell count, hematocrit and serum protein in four dogs (19 experiments) and in three of the same dogs after splenectomy (11 experiments).

To explore these possibilities, acute experiments were done under anesthesia. After removing the spleen of a dog, concentrated blood from another dog was injected at intervals, in an attempt to simulate experimentally a splenic contraction. The donor blood was first centrifuged, most of the serum discarded, and the concentrated residue injected. Samples of blood were drawn ten minutes after each injection and the previously described determinations (red cell count, hematocrits and serum proteins) were carried out. Corrections were made for the small amount of protein injected.

Table 2 gives the results of these experiments. In every case, an absolute increase in formed elements, produced experimentally, caused a

concentration of serum proteins. This concentration appears to be approximately proportional to the increases in hematocrit reading, though a quantitative relationship has not yet been determined.

TABLE 2

Results of six acute experiments in which blood, concentrated in red cells, was transfused at intervals in splenectomized dogs, indicating the compensatory concentration of serum with absolute increases in cell volume

DATE	WEIGHT	BLOOD INJECTED	DONOR BLOOD	HEMATOCRIT	INC.	RBC	INC.	SERUM PROT.	PROT INC.
	kgm.	*cc.*	*per cent cells*	*per cent cells*	*per cent*	*million*	*per cent*	*gr./100*	*per cent*
1/23/38	7.8	Normal		50.0		6.54		6.23	
		50.0	87.0	55.3	10.6	7.60	16.0	6.60	6.0
1/28/38	10	Normal		62.0		7.52		6.65	
		50.0	89.0	66.5	7.2	8.05	7.0	6.83	2.7
		50.0	89.0	70.5	13.7	9.53	26.7	7.28	9.5
2/ 1/38	20	Normal		55.3		7.44		6.60	
		25.0	81.0	61.2	10.6	7.99	7.4	6.86	3.9
2/ 9/38	14	Normal		50.0		7.90		6.28	
		25.0	80.0	56.3	11.2	8.49	7.2	6.45	2.8
		25.0	80.0	58.2	16.2	9.99	26.2	6.53	4.0
		25.0	80.0	61.0	22.0	10.95	38.0	6.60	5.0
		25.0	80.0	63.0	26.0	11.45	42.0	6.60	5.0
2/16/38	7.8	Normal		50.5		7.36		6.13	
		50.0	95.0	54.4	7.7	8.09	9.9	6.75	9.8
		50.0	95.0	55.6	10.1	8.76	18.8	6.96	13.7
2/24/38	10	Normal		46.4				5.66	
		100.0	97.0	55.6	19.9			6.26	10.4
		50.0	97.0	61.6	32.9			6.49	14.4
		50.0	97.0	66.3	42.9			6.79	19.9

DISCUSSION. The stimulus for the splenic contraction is unknown. However, Barcroft and Elliot (5), working on animals in heated chambers, observed contractions in normal spleens but none in denervated spleens. These results suggest a nervous mechanism. To compensate for the internal heat, there is a vasodilatation of the skin vessels in order to cause loss of heat by conduction (11). Thus, there is a shift of blood from the viscera to the periphery, which might account for the contraction of the spleen. It is also possible that the increased metabolism of the animal and the greater rate of circulation causes an "oxygen want" which might stimulate contraction.

There seems to be no alternative to the conclusion that the spleen is

directly responsible for the red cell concentration changes observed in these short duration fever treatments of dogs; and further, that this organ is, partly, or entirely, indirectly responsible for the concentration of plasma. The increase in serum protein with fever in normal animals and the absence of this increase in splenectomized animals, together with the results of the acute experiments cited above, tend to show that the addition to the circulation of concentrated red cells from the spleen has caused a displacement of fluid from the serum, possibly in order to maintain a constant blood volume.

These results, however, by no means rule out the possibility that the spleen is liberating concentrated plasma as well as red cells. Experiments in which splenic vein blood and splenic artery blood were drawn simultaneously before and after splenic contractions showed such insignificant changes in serum proteins that these experiments were discontinued. However, a recent paper by Gorecsky and Ludany (11) shows that when all the vessels of the spleen except the splenic vein are tied off, and the splenic vein cannulated, contractions induced by faradic current applied directly to the spleen cause the liberation of blood containing plasma much higher in total nitrogen than the plasma of peripheral blood. The five experiments cited in their paper show an average increase in total nitrogen of 12.3 per cent over the normal. This result means that if the spleen liberated blood totaling one-third of the total blood volume (Barcroft), and if this blood consisted of 75 per cent cells and 25 per cent plasma, the addition of the serum liberated by the spleen would cause an increase in circulating serum of approximately 3–4 per cent. Therefore, even if this phenomenon occurs, it is not enough to explain the 10 per cent increases noted in our experiments. Thus, the mechanism most significant in the production of the blood changes there noted still seems to be the concentration of blood plasma resulting from the influx of stored erythrocytes expelled by the spleen.

CONCLUSIONS AND SUMMARY

1. Artificial fever induced in dogs by a G. E. inductotherm causes a significant contraction of the spleen.

2. This contraction is responsible for the observed polycythemia and the blood plasma concentration since neither of these effects occur in splenectomized animals.

3. Evidence is presented to show that absolute increase of red cells, produced experimentally, in acute dogs, by transfusion of blood corpuscles, results in increase of serum protein, due possibly to loss of water into the tissues.

It has been a great opportunity to carry out this work under the direction of Dr. A. B. Luckhardt.

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INFLUENCE OF EXERCISE ON BLOOD PRESSURE, PULSE RATE AND CORONARY BLOOD FLOW OF THE DOG

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There are many valuable studies concerning the effect of exercise on the pulse rate and blood pressure of man (Bock and Dill, 1931), but the literature on the influence of muscular exercise on the blood pressure and pulse rate of the dog is relatively meager. The report of Samaan (1935) is the only recent work dealing with this subject that we have encountered. Consequently, it seemed desirable to study the effect of exercise on the blood pressure and pulse rate of the normal dog as well as to investigate the relation of these factors to coronary blood flow.

Before this work was begun it was considered important to determine whether the presence of the thermostromuhr unit for measuring blood flow in the coronary artery affected the various aspects of cardiac function. In order to conserve space the results of these experiments are here briefly summarized.

If the unit interfered with the nutrition, conduction or rhythm of the heart this should be revealed by the electrocardiogram. If the work capacity of the heart were seriously impaired it would probably be detectable in the records of the blood pressure. The presence of the unit did not produce alterations in the electrocardiogram of the dog greater than resulted when a similar control operation was done and the unit omitted (fig. 1). The preoperative blood pressure in the femoral artery obtained with the technic of Hamilton, Brewer and Brotman was not significantly different from that seen postoperatively whether or not the unit was present on the coronary artery (table 1). Since by the methods of determination employed the presence of the thermostromuhr unit did not cause an alteration in the nutrition, conduction, rhythm or work capacity of the heart, the experiments described in the remainder of this report were done with the assurance that the results should closely approximate what occurs in the normal animal.

METHODS. The dogs used in these experiments were trained to walk and run on a treadmill. When the animal was sufficiently trained the femoral

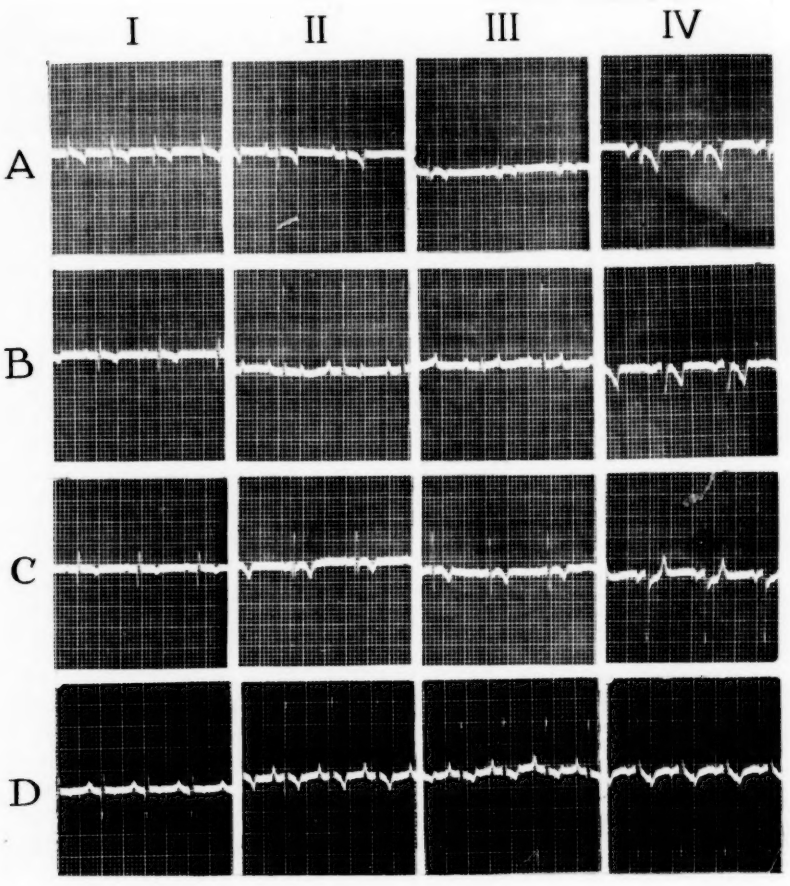

Fig. 1. A series of electrocardiograms of the dog taken before and after placing the thermostromuhr unit on the circumflex branch of the left coronary artery. In addition to the conventional leads a chest lead was used. This consisted in placing the right electrode at a point a few centimeters to the left of the ventral midline and over the fourth intercostal space. The left electrode was placed a few centimeters to the left of the dorsal midline and just posterior to the dorsal margin of the scapula. To insure uniformity in placing the electrodes for successive electrocardiograms the points of application of the electrodes to the skin were marked by tattoos with India ink. A: One hour before operation. B: Four hours after operation. C: Twenty-four hours after operation. D: Four days after operation.

artery was cannulated under local infiltration anesthesia. The blood pressure and pulse rate and in a few experiments the rate of respiration

were recorded by standard methods—mercury manometer, kymograph and smoked drum. The manometer was damped to record approximately the mean blood pressure. The same technic for recording blood pressure was used in our studies of coronary flow, blood pressure and pulse rate, the

TABLE 1

Pulse rate and blood pressure before and after application of thermostromuhr unit to coronary artery compared with findings in control animals where unit was omitted

DOG	WEIGHT	PREOPERATIVE DAYS	POSTOPERATIVE DAYS	PULSE RATE	BLOOD PRESSURE		BLOOD FLOW, AVERAGE
					Systolic	Diastolic	
	kgm.				*mm.* Hg	*mm.* Hg	*cc. per minute*
1	18.4	2		105	209	105	
			$\frac{1}{1\cdot2}$	144	173	91	104
			$\frac{3}{1\cdot2}$		210	104	75
			2	114	204	100	59
			4	125	205	108	58
			5	125	195	93	48
2	16.0	9		69	169	96	
		2		56	182	99	
			1	168	178	105	43
			2	84	170	102	54
			5	55	150	89	42
			6	70	177	98	42
			8	50	175	104	48
3*	12.0	8		130	218	132	
		5		110	231	130	
		1		150	205	120	
			2	170	208	116	
			5	131	197	114	
			7	168	188	107	
4*	15.5	8		90	196	117	
		5		100	195	104	
		1		100	183	103	
			2	140	187	108	
			5	94	222	125	
			7	116	197	116	

* Control—thermostromuhr unit was not placed on the coronary artery but the operative procedures necessary for doing so were done.

thermostromuhr unit having been previously applied to the circumflex branch of the left coronary artery under a general anesthetic and with sterile technic as described in a previous publication (4). The experiments were then done after sufficient recovery from the operation.

RESULTS. *The effect of exercise on mean blood pressure and pulse rate in the normal dog.* In a series of observations on normal dogs weighing from 9.8 to 20.0 kgm. the resting mean blood pressure varied from 108 to 166 mm. of mercury. When the animals were exercised with the treadmill horizontal, the work done was not determined and the increase in the mean blood pressure varied from 10 to 17 per cent. When the treadmill was elevated so that the rate of work varied from 97 to 292 kgm. m. per minute the increase in blood pressure varied from 24 to 50 per cent over the value at rest. The percentage increase in blood pressure was not proportional to the rate of work when all the animals were considered since one dog doing work at the rate of 97 kgm. m. per minute had a 40 per cent

TABLE 2

Effect of muscular exercise on the blood pressure and pulse rate of the dog

DOG NO.	WEIGHT	MEAN BLOOD PRESSURE					PULSE RATE PER MINUTE					RATE OF WORK, WITH T. M. ELEVATED
		Resting	T. M. horiz.* (max. B. P.)	Per cent incr. over rest	T. M. elevated (max. B. P.)	Per cent incr. over rest	Resting	T. M. horiz.* (max. P. R.)	Per cent incr. over rest	T. M. elevated (max. P. R.)	Per cent incr. over rest	
	kgm.	*mm.* Hg	*mm.* Hg	*mm.* Hg	*mm* Hg	*mm.* Hg						*kgm./ min.*
1	20.0	120	140	17	180	50	74	156	111	165	123	198
2	12.0	166			206	24	140			180	29	211
3	9.8	120	132	10	168	40	72	100	39	150	108	97
4	16.6	142	156	10	206	45	72	165	129	180	150	292
5	13.4	108	124	15	145	34	90	180	100	190	111	132
6†	26.5	118	132	12	158	34	152	166	9	176	16	262
7†	11.8	106	120	13	136	28	126	187	48	176	40	75
8†	11.8	112			142	27	136			220	62	122
					158	41				216	59	

* When the treadmill is horizontal, the work done is not determined.

† Thermostromuhr units on circumflex branch of left coronary artery.

increase while another doing work at the rate of 211 kgm. m. per minute had an increased blood pressure of only 24 per cent above the resting level. However, the percentage increase in the blood pressure of a given animal definitely reflected the rate of work (table 2). In each dog successive increases in the rate of work during continuous exercise resulted in a rise in blood pressure above the previous level. Usually each decrement in the rate of work was followed more or less promptly by a decrease in the blood pressure.

In our series there was a marked variability in the behavior of the blood pressure as exercise was continued. In every experiment the blood pressure responded rapidly to the onset of work and to changes in its rate but as the work progressed with some dogs the pressure attained its maximum

in from one to three minutes where it remained until the rate of work was changed (fig. 2). In other cases the blood pressure fell below the maximum but rose again to near the maximal pressure as the work continued at a given rate. In another experiment the blood pressure continued to rise until the rate of work was altered. Our results were less consistent than those reported by Gillespie, Gibson and Murray for man. These workers found that as a given rate of work was continued the maximal blood pressure was reached early in the period of exercise after which it fell gradually and irregularly to a lower level. Likewise Samaan working with dogs reported a temporary rise of blood pressure in the carotid at the onset of exercise with a decline in blood pressure as work was continued and upon cessation of exercise, an abrupt fall to a subnormal level.

The resting pulse rate of one dog was 140 per minute. That of the others was from 72 to 90 per minute. The change in pulse rate was usually large

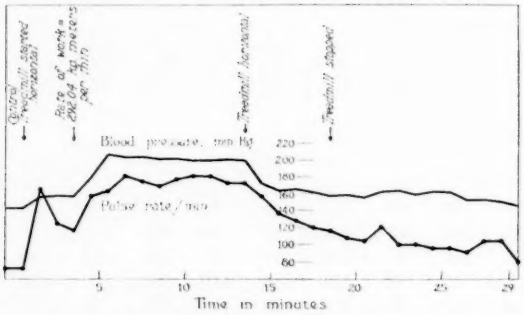

Fig. 2. Graphs of mean blood pressure in the femoral artery and graphs of pulse rate showing response to different rates of work.

at the beginning of exercise regardless of the rate of work. In one dog, whose resting pulse rate was already high, the increase in pulse rate was only 29 per cent when the rate of work was 211 kgm. m. per minute. In the remaining dogs the maximal increase in pulse rate, with one exception, was almost as great when the dogs were running with the treadmill horizontal as when work amounting to as much as 292 kgm. m. per minute was being done. For example, one of the dogs doing work at the rate just mentioned had an increase in pulse rate over the resting level of 150 per cent while the increase over the resting level when the treadmill was running horizontal was 129 per cent. It may be seen that changes in blood pressure are more nearly proportional to the rate of work than are the changes in pulse rate. As a rule the pulse increased at a more rapid rate than did the blood pressure at the beginning of work and declined more rapidly than the blood pressure when the rate of work was decreased or stopped. The maximal

pulse rate for a given rate of work was usually reached within one to three minutes after the exercise started. Frequently the pulse rate fell considerably below the initial rise and remained relatively constant at the lower level.

These findings on the dog are in striking contrast with those reported for man by previous workers, who have found that the pulse rate continues to increase throughout the period of exercise until the maximal pulse rate is reached. Overshooting or an initial rise above the pulse rate that is subsequently maintained is more frequent when exercise is begun than with the successive increments of work. In our experiments overshooting was not a pronounced characteristic of the behavior of the blood pressure at the beginning of exercise or when the rate of work later was increased.

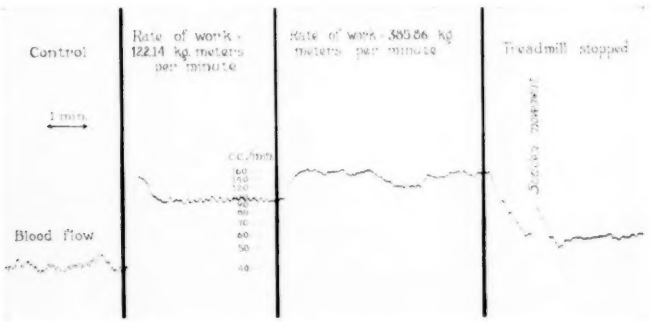

Fig. 3. Photographic record of blood flow in the circumflex branch of the left coronary artery of a dog before, during, and after exercise at different rates on a treadmill.

Previously (4) we have reported the changes in blood flow in one of the coronary arteries resulting from exercise on a treadmill. Characteristically, the blood flow increased rapidly with the beginning of exercise and subsequently with each increase in the rate of work. However, as the dog continued to do work at a given rate the flow usually fell from the peak reached during the first few minutes of exercise to a lower level, near which it remained until the rate of work was altered (fig. 3). Since it is known that changes in blood pressure are chiefly responsible for changes in the coronary blood flow of the denervated heart of the heart-lung preparation (Markwalder and Starling, 1913; Anrep and Segall, 1926) it was thought probable that an explanation of the observations on coronary blood flow just described would be found in a study of the blood pressure and coronary blood flow recorded simultaneously in the trained dog. Therefore in addition to the data obtained on the blood pressure and pulse rate of the normal dog, studies were made on the effect of exercise on dogs to whose coronary arteries thermostromuhr units had been previously applied. A

series of dogs was studied from this point of view and representative data from these experiments are here presented.

Simultaneous observations on blood pressure, pulse rate and coronary blood flow. The effect of muscular exercise on the blood pressure and pulse rate of dogs to whose coronary arteries thermostromuhr units had been

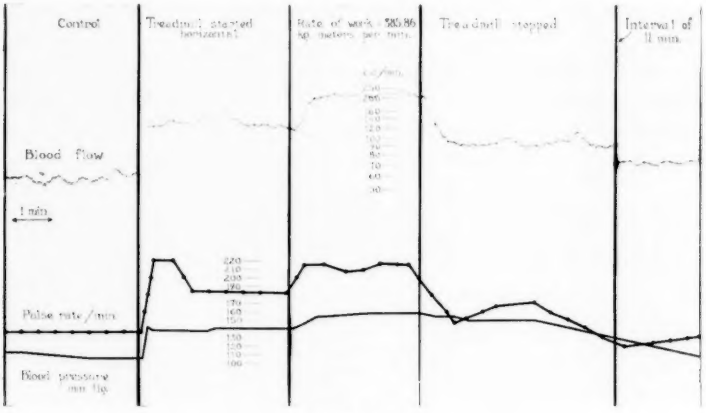

Fig. 4

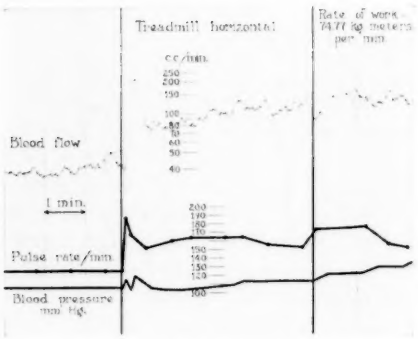

Fig. 5

Figs. 4 and 5. From above downward: photographic record of blood flow in the circumflex branch of the left coronary artery; graph of pulse rate and graph of mean blood pressure simultaneously recorded before and during exercise on a treadmill.

applied was in every respect comparable to what has just been described for the control animals (figs. 4 and 5). The average resting pulse rates of the control group were 72 to 140; those of the animals with thermostromuhr units on their coronary arteries were 126 to 152 (table 2).

The results of the present series of experiments with respect to coronary

blood flow were similar to those previously reported (fig. 3). When exercise was begun the coronary blood flow rapidly increased but overshooting did not always occur (fig. 4). The maximal flow was sometimes main-

TABLE 3

Influence of rate of work on average blood flow in circumflex branch of left coronary artery of the trained dog

EXPERIMENT	RATE OF WORK*	BLOOD FLOW			REMARKS
		Control	At beginning of work	As work progressed	
	kgm. m. per minute	*cc. per minute*	*cc. per minute*	*cc. per minute*	
		43			
1	Treadmill horizontal:		90	72	
	99		90	80	
	311		130	120	
		44			
	Treadmill horizontal:		100	78–57	Extra exertion; dog barked and strained at leash
2	99		80	71	
	311		86	86	
	99		86	72	
	Treadmill horizontal:		72	60	
		41			
3	311		127	90	
		40			
4	122		150	95	
	386		160	140	
		60			
5	122		255	188	Extra exertion
	386		278	250	
		37			
	Treadmill horizontal:		65	52	
6	234		80	65	
	311		80	60	
	Treadmill horizontal:		72	44	

* When the treadmill is horizontal the work done is not determined.

tained throughout the initial period of work and subsequent increases in the rate of work resulted in further rapid augmentation of the coronary blood flow which might be maintained or the flow might decline to a lower level as the increased rate of work was continued (fig. 5). The greatest

increase in coronary blood flow observed with the rates of work employed amounted to about 400 per cent. The details of the influence of the rate of work on the coronary blood flow in a series of representative experiments are shown in table 3. Since the rate of respiration is so variable in the dog it is of little value as an indicator of the rate of work.

COMMENT. On the basis of the results obtained by previous workers on man and the dog it was logical to assume that the increased coronary blood flow occurring at the onset of exercise and accompanying successive increments of work was largely due to elevations of blood pressure. However, when the data on the blood pressure, pulse rate and coronary blood flow recorded simultaneously were studied it was obvious that the large increases in coronary blood flow occurring at the onset of exercise and with later increments of work could not be satisfactorily accounted for on the basis of increases in blood pressure alone. The relatively slight increases in blood pressure that were frequently seen at the onset of exercise with the treadmill horizontal could hardly be responsible for the large increases in coronary blood flow that invariably occurred (fig. 5).

When the changes in pulse rate that accompany the onset of exercise and the successive increments in the rate of work were studied it was found that the changes in pulse rate mirrored surprisingly well the augmentation in the coronary blood flow. There is no doubt that fluctuations in blood pressure influence the volume of coronary blood flow in the trained dog but there are apparently other factors as important as blood pressure in this regard.

SUMMARY AND CONCLUSIONS

A series of experiments has been done on control dogs and dogs in which thermostromuhr units had been previously applied to the coronary arteries in an effort to determine the effect of exercise on the pulse rate, blood pressure and coronary blood flow. Each animal had been accustomed to perform work at different rates on a treadmill.

Preliminary experiments demonstrated that the presence of the coronary thermostromuhr unit had no significant effect on the electrocardiograms or blood pressure of the animals studied.

Successive increases in the rate of work produced additional rises in blood pressure which were roughly proportional to the rate of work for the individual animals in both the control and experimental groups. As a given rate of work continued the behavior of the blood pressure varied from animal to animal. In certain experiments the blood pressure reached a maximal level early and declined, in some it remained near the maximum, while in others it continued to augment throughout the period of exercise.

The pulse rate of the dog is a less reliable index of the rate of work than the blood pressure. The maximal pulse rate with low rates of work may be as great initially as with higher rates of work.

Simultaneous records of pulse rate, blood pressure and coronary blood flow were made during exercise. The marked increases and decreases of coronary blood flow observed with various increments and decrements of work could not be satisfactorily accounted for by changes in blood pressure alone. The change in pulse rate with exercise was usually a better criterion than the blood pressure of changes in coronary blood flow. The evidence indicates other influences as important as blood pressure in augmenting coronary blood flow when a trained dog is given successive increments in the rate of work.

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